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BIOLOGICAL BULLETIN.

NOTES ON MEROGONY AND REGENERATION IN RENILLA.

EDMUND B. WILSON.

H. B. Torrey[1] has published a brief note on regeneration in *Renilla* in which he shows that the young colonies possess a high regenerative capacity and records some valuable preliminary observations, but owing to the difficulty of procuring sufficient material he was unable to carry these observations far enough to reach a decisive result on some of the important questions concerned. During the summer of 1902, after repeated failures in preceding years, I was fortunate enough to secure at Beaufort, N. C.,[2] four lots of fertilized eggs from which hundreds of young colonies were reared (they were kept alive three weeks but produced only the two primary buds) and also a considerable number of older but still young colonies, obtained from the sand. I was thus able considerably to extend Torrey's experiments and also to make observations on the development of egg-fragments that yielded some suggestive results.

(*a*) The Development of Egg Fragments.

All efforts failed to fertilize the eggs artificially, so that only fertilized eggs were available for experiment, but these can easily be cut individually with the scalpel into two or more pieces. As I showed in my paper of 1882[3] the cleavage-nucleus divides several times (from three to five) before cleavage of the cytoplasm occurs, the egg usually segmenting, from two to three hours after it is laid,[4] at once into eight or sixteen blastomeres

[1] "Some Facts Concerning Regeneration and Regulation in *Renilla*," Biol. Bull., Vol. II., p. 6, 1901.

[2] I am indebted to the Hon. G. M. Bowers, U. S. Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort laboratory, and to Dr. Caswell Grave, director of the laboratory, for his kind coöperation.

[3] "The Development of *Renilla*," *Phil. Trans.*, Vol. III., p. 24.

[4] As I found twenty years ago the eggs are always laid between 5:30 and 6 A. M.

(exceptionally into a larger number), though with many variations. Twenty-four eggs were cut, at intervals of three to ten minutes during the period between discharge and cleavage, into from two to five pieces that were isolated in water containing spermatozoa. Of these fragments at least one piece from each egg developed in all but one case. Of the first nineteen, covering a period of eighty-seven minutes only one piece from each egg developed (eighteen cases). These fragments divided like the whole eggs, and at the same time with the latter (two and one half hours), into approximately eight or sixteen blastomeres. Of the five remaining cases two or three pieces from each egg developed, segmenting in such a way that the total number of blastomeres formed from each egg was at least approximately, and probably accurately, the same as those produced from an entire egg. The number of the blastomeres in the segmenting fragments of the respective eggs (which, owing to lack of time, could not be exactly counted) were approximately 4 + 12, 4 + 12, 4 + 8, 4 + 5 + 16, 4 + 12. These results are what would have been expected. It is evident that the development of only a single piece in the earlier period is due to the fact that the egg still contains only a single nucleus; and only when the nucleus has divided one or more times does the possibility arise of obtaining more than one nucleated piece from a single egg. The facts seem worth recording, as showing that, despite the absence of a fertilization-membrane after fertilization has occurred, non-nucleated pieces of the egg cannot be again fertilized (which agrees with my later experiments on *Cerebratulus*, an account of which is now in press); and also that the period at which cleavage occurs does not depend on the number of nuclei in the piece but on some other progressive change which reaches the critical point at the same time whether the egg be cut to pieces or remain a whole.

Of more general interest is the fact that egg fragments not more than one fourth the bulk of the egg may develop into dwarf larvæ, which, after swimming for the normal period (about forty-eight hours), sink to the bottom, develop tentacles, and *produce the first pair of buds in normal fashion.* Thus arise dwarf colonies of various sizes that are, in every detail, miniatures of the normal colonies of the same age. Fig. 1 shows side

by side three colonies eight or nine days old, the largest being he product of a whole egg, the smallest that of a fragment about one fourth the size of a whole egg, and the third that of half an egg. These drawings are from camera sketches that show very nearly the true proportions, though owing to the occasional movements of the animals the details had to be drawn free hand. Allowing for slight differences in the state of expansion it may

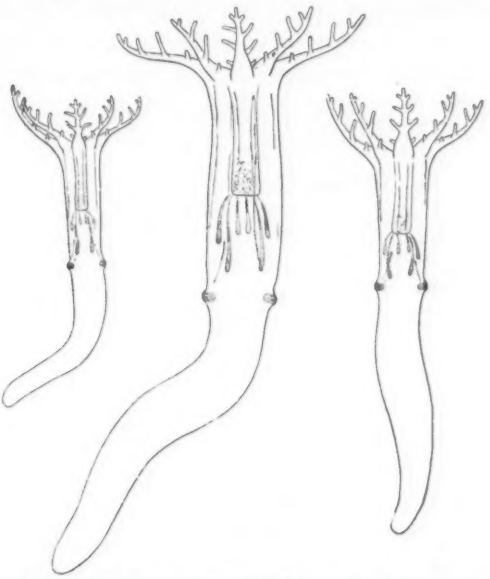

FIG. 1. In the middle, primary polyp with first pair of buds, reared from an entire egg, eight days. To the right and left are dwarf colonies nine days old drawn to the same scale, from fragments of fertilized eggs, one of approximately one half, the other of one fourth the bulk of an entire egg.

be seen that the dwarfs are very exactly similar to whole colonies of reduced proportions. Beyond this stage it was not found possible to rear the young colonies, even after three weeks, probably owing to the difficulty of providing the animals with suitable food.

These results are of some interest as proving that in this case the process of budding does not depend on the attainment of a certain size-limit by the parent stock, but on that of a definite ontogenetic stage, exactly as if the buds were organs of a single individual. It can hardly be doubted that in the ancestral type

the buds were set free from the stock, as still occurs in many polyps. Here, however, their individuality has become completely merged in that of the organism as a whole, which develops, as it behaves in the adult condition, essentially as a unit, the bud-formation in the merogonic development being subject to a process of regulation in a manner precisely analogous to the formation of organs in the development of a dwarf pluteus or pilidium from an egg fragment. This conclusion, which is also reached from the facts of regeneration described beyond, is not without a broader interest in its bearing on the possible derivation of metameric animals from linear colonies, or even on the relation of Metazoa to colonial Protozoa.

(*b*) Development of Fragments of Planulas.

I made a few experiments by cutting to pieces the spherical planulas of two to four hours (consisting of 128 cells or more) which are placed on record as suggesting the interest of more extended studies of the same kind. Like fragments of the unsegmented eggs, the planula fragments quickly round out and continue their development for a time apparently unimpaired, and in this way were obtained from a single egg several swimming planulas — in one case nine from a single egg, in one case six, in two cases five, and two or three from the remainder. Though only seven planulas were thus operated the results seem to show that at this period the power of regulation is already somewhat diminished. Of the thirty-one fragments obtained only two developed into normal dwarf colonies. All the others produced abnormal or defective larvæ, the most abnormal ones, as was to be expected, arising from the smaller fragments. Some failed to form stomodæum or septa, others produced stomodæum and the normal number of mesenteries, but no peduncle, several were nearly normally formed but produced no buds, and only a single pair of mesenterial filaments, one produced only a single large median bud, while several of the smaller larvæ showed less than the normal number of mesenteries. Of the nine fragments of a single planula six ultimately died, but the remaining three pieces, including the two largest, developed into larvæ all of which possessed stomodæum and mesenteries. The smallest

of these formed no tentacles, peduncle or mesenterial filaments, and only four mesenteries. Both the other two developed six mesenteries and rudimentary tentacles, one pair of short mesenterial filaments; only one of them formed a peduncle. This case indicates that the number of mesenteries is not specified at this period; for although none of the larvæ produced the full number the total number formed from the original egg was sixteen.

(*c*) Experiments on Regeneration in the Young Colonies.

My principal object in studying the young colonies was to examine the relation between morphallaxis and neomorphosis (to adopt Morgan's terms) for which purpose such an organism as *Renilla* offers obvious advantages, and also to ascertain, if possible, how far the process of bud-formation is capable of regulation. The main results reached by Torrey were: (1) That the polarity of the primary polyps was never reserved, a polyp always regenerating at the anterior end of a piece and a peduncle at the posterior end; (2) that the power of regeneration was confined to the budding zone; (3) that after oblique section, a remoulding of the old parts occurred by a regulation "in a plastic fashion," but he did not succeed in following the later changes long enough to ascertain whether this plastic remoulding was permanent, and involved the establishment of a new axis of symmetry. Like Torrey, I found that when the peduncle was removed, at any level, a new peduncle very quickly reappeared; that removal of the axial polyp by transverse section led to regeneration of that polyp; and that as a rule a severed peduncle did not regenerate a new axial polyp at the anterior end; and that by oblique section through the budding zone two colonies might be produced from one. On some points, however, my results differ from his, while they give a pretty definite answer to the undecided question regarding the phenomena of remoulding.

1. In a single case a reversal of polarity was obtained from a colony having five buds that was cut into three pieces by sections anterior and posterior to the budding zone as shown in Fig. 2, *A*. As a rule after operations of this type, only the middle piece, containing the budding zone, regenerates a perfect colony,

a new peduncle being formed posteriorly and a new axial polyp anteriorly, while neither the severed axial polyp nor the peduncle regenerates, though both may live for a sufficient period of time (in some cases a week or more). In this instance the peduncle, as usual, failed to regenerate, while the middle piece regenerated an axial polyp in front and peduncle behind. The anterior piece (2 *B*) formed an exception to the rule in that it regenerated a large polyp at the posterior end, a form being produced with two similar polyps united at the base and point-

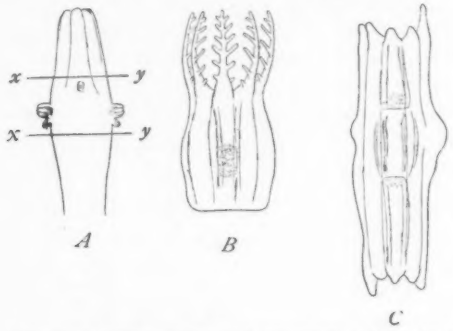

FIG. 2. *A*, outline of young colony with two pairs of buds, in the contracted state, showing planes of section. *B*, the anterior piece (axial polyp), twenty-four hours later; the middle piece already showed a new axial polyp and peduncle well under way. *C*, the anterior piece fifteen days after the operation, with new polyp at base, and the original tentacles reduced.

ing in opposite directions (Fig. 2, *C*). Each possessed a stomodæum, and the mesenteries were continuous from one to the other, bearing six mesenterial filaments. At one side was a rounded elevation that may have been a regenerating peduncle; but the animal died without further change. An interesting feature of this case was the degeneration of the original tentacles, which lost their pinnules and became greatly shortened so as to form rudiments closely similar to those of the regenerated polyp, or those of an early larva. I have observed the same phenomenon in young colonies in which the peduncle, after its removal and regeneration, had been again successively twice removed and regenerated, the whole animal having been in the meantime considerably reduced in size. This shows that regeneration takes place at the cost of material throughout the colony, even involving regressive

changes in parts already completely formed. Interesting results can doubtless be obtained by the further study of these changes.

2. The above case not only shows a reversal of polarity but proves that regeneration may take place anterior to the budding zone. A similar result was also given in several cases where the peduncle was removed by section posterior to the budding zone; but this only occurs when the section is not far removed from the budding zone.

In at least one such case it is certain that the section was outside the normal limits of the budding zone, the colony having

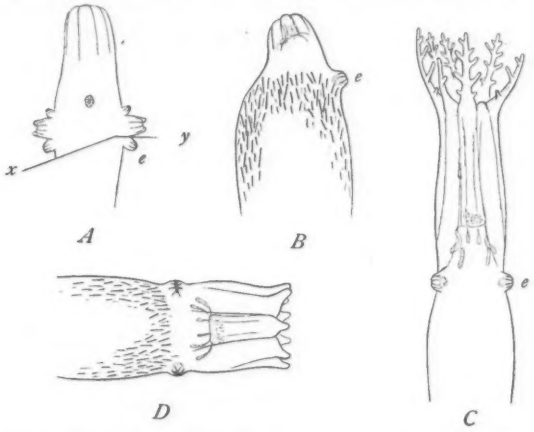

FIG. 3. *A*, outline of young colony with three pairs of buds, showing plane of section. *B*, posterior piece after forty-eight hours, with new axial polyp forming; a new peduncle had formed on the anterior piece. *C*, the same nine days later, with nearly perfect axial polyp and new bud on the left side. *D*, new axial polyp and pair of buds regenerated from the anterior end of a peduncle, cut off behind the budding zone from a young colony with seven pairs of buds; five days after operation.

seven buds on each side. In two days a new axial polyp was well under way at the anterior end of the severed peduncle. In five days this polyp was well developed, with short tentacles and mesenterial filaments, while a pair of symmetrically placed buds had appeared in the same position as in the young colonies (Fig. 3, *D*). A condition was thus attained essentially similar to that of the young colonies developed from the egg (*cf.* Fig. 1), though the peduncle was of course greatly exaggerated in

relative size. This colony died eight days after the operation without further change except further development of the axial polyp.

3. Experiments to test the degree of specification of the persons of the polymorphic colony, and the relation between morphallaxis and neomorphosis, while not absolutely conclusive, give strong ground for the conclusion that, despite a limited power of heteromorphosis and regulation, as shown by the foregoing experiments and those of Torrey, the individual persons are on the whole definitely specified in respect both to the rate of growth and to their axial relations to the colony as a whole. To test this I first tried to see whether the removal of the large buds would result in the more rapid development of the remaining small buds. For this purpose colonies were cut in such a way as to leave only a single small bud attached to the peduncle (Fig. 3 *A*). Since this bud (*e*) is destined to develop into a polyp like the axial one it might have been expected that it would develop at once into a new axial polyp. The fact is quite to the contrary, for the small bud remains stationary while a new axial polyp is produced at the front end of the peduncle (Fig. 3, *B*, *C*). In one case, indeed, the small bud completely disappeared during the process, being apparently absorbed, the whole growth-energy having concentrated in the regenerative process. In the individual figured the small bud remained wholly unchanged while a new axial polyp was regenerated (slightly abnormal in respect to the tentacles), but in the meantime a second bud was formed in the corresponding position on the opposite side of the body; so that the young colony attained the same condition (Fig. 3, *C*) as in Fig. 3, *D*. The comparison of these two cases is interesting, since the same condition was obtained by different methods, both buds being formed anew in the one, while in the other only one bud was produced to form the fellow of the existing one. This indicates that the characteristic first stage in the normal development, with a single pair of buds (Fig. 1) is a definite ontogenetic stage that represents, as it were, a condition of equilibrium that is first restored after the operation before a second step is taken. A definite morphological relation therefore exists between the persons of the

colony that seems exactly comparable to that existing between the different organs of a single individual — essentially the same result as that given by the formation of dwarf colonies from egg fragments.

I endeavored to see whether, by removing all the buds except the exhalent zoöid, this rudimentary person could be made to

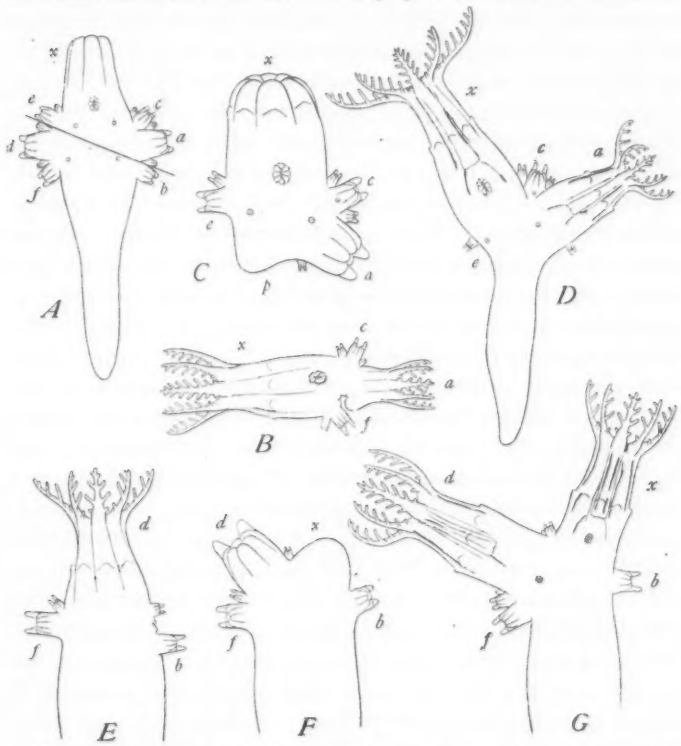

FIG. 4. Regeneration after oblique section. *A*, outline of colony (contracted) with six pairs of buds, showing plane of section. *B*, anterior piece, four hours after operation; after twelve hours the wound had entirely closed. *C*, the same forty-eight hours after operation, showing rudiment of peduncle. *D*, the same five days after operation; return of lateral bud to its original position. *E*, posterior piece, twenty-four hours after operation; lateral bud in the former position of the axial polyp. *F*, the same twenty-four hours later; new axial polyp (*X*) forming. *G*, the same, seven days after operation; new axial polyp well developed, with new exhalent zoöid; return of lateral bud nearly to its original position. During the following ten days the axial polyp became as large as the lateral one, but no other buds were formed.

develop into a fully developed polyp. None of these cases lived more than six days, but in one of them the exhalent zoöid had very considerably enlarged. This may, however, have been a merely passive expansion, and I regret that no decisive result was reached. Repetition of Torrey's experiment of cutting the colony diagonally across at an angle of 45° gave a suggestive but not quite conclusive result. The best case obtained is shown in Fig. 4, the section being in such a plane as to leave one of the primary lateral buds (*a*, *d*) in each piece, the posterior piece having in addition four smaller buds and the anterior one six. Within an hour after the operation, and while the wounds were still widely open, a complete readjustment had occurred in the relative position of buds, and after twelve hours the wounds were entirely healed. The large lateral bud (*d*) of the posterior piece was so displaced as to be directed straight forward, giving exactly the appearance of an axial polyp (Fig. 4, *E*), while by a corresponding process in the anterior piece the lateral bud (*a*) was directed straight backward (4, *B*). In the course of 48 hours however a rapid formation of new tissue took place in both pieces, forming the beginning of a new axial polyp in the posterior piece (4, *F*) and of a new peduncle in the anterior one (4, *C*). At the end of seven days the new axial polyp of the posterior piece (4, *G*, *x*) was fully formed though still not quite as large as the original lateral one, with four pairs of tentacular pinnules, and a median dorsal bud had appeared exactly in the position of the exhalent zoöid. When the colony was fully expanded the new axial polyp was directed almost straight forward while the original lateral bud (*d*) was swung quite over to one side, nearly in its original position, though the colony still showed a very marked asymmetry. At the end of seventeen days the axial polyp was as large as the lateral one, but no other essential change had occurred. The colony was at this time killed for preservation, since I was compelled to leave Beaufort; but the evidence of the specification of the buds obtained from this and the other cases described renders it probable that the original condition would ultimately have been restored and that the primary process of moulding would in the end have been wholly overcome by the regenerative process. In the meantime

the anterior piece (4, *D*) had generated a long peduncle, from the anterior end of which the original axial polyp and right lateral one diverged as shown in the figure.

This case shows with great clearness that very soon after the operation the fragment is plastically remoulded, in a manner somewhat similar to that described by Hargitt and Morgan,[1] in the medusa *Gonionemus*, so that without the formation of new tissue the pieces assume a new condition of equilibrium which in the posterior piece brings a lateral polyp into the former position of the axial one. This process takes place so quickly that it seems inadmissible to suppose that it is due to an active process of growth. It gives rather the impression of a mechanical process due to the operation of purely physical factors (tension of the tissue or the like) by which a new condition of equilibrium is restored as nearly as possible like that of an entire colony, and hence representing a case of pure "mechanical regulation" in the sense in which Child has construed the primary process of morphallaxis in planarians.[2] Broadly speaking this process is probably of the same nature as that by which the edges of a cut surface close, though in both pieces the change of form was already complete while the wound was still widely open, as may be seen from Fig. 4, *B*. As such, it cannot be considered as part of the regenerative process in the strict sense,[3] indeed this is proved by the fact that the ensuing regeneration gradually counteracts the effect of the initial remoulding. I can, however, find no ground in Morgan's own discussions for excluding such a mechanical remoulding of the old parts from the conception of morphallaxis in general, and the same ground is taken in the case of *Stenostoma* by Child, who reaches the conclusion that morphallaxis in this form is "essentially a change in form resulting from differences in mechanical tension in the piece as compared with the whole" ('02, p. 414). The facts of initial morphallaxis observed in *Renilla* fall in very well with Morgan's tension-hypothesis, and

[1] C. W. Hargitt, "Recent Experiments on Regeneration," *Zoöl. Bull.*, I., 1897. T. H. Morgan, "Regeneration in the Hydromedusa *Gonionemus*," *Am. Nat.*, XXXIII., 1899.

[2] C. M. Child, "Fission and Regulation in *Stenostoma*," *Arch. Entwm.*, XV., 2, 3, 1902.

[3] *Cf.* Morgan, "Regeneration," p. 69.

the remarkable quickness of the process is doubtless due to the extremely plastic character of the tissues in the animal, ordinarily shown in its frequent and very marked changes of form. In planarians the initial remoulding takes place much more slowly and forms an initiative for other regulative changes by which the piece is permanently remodelled into a new form. In *Renilla* such regulative changes are apparently absent, or present in only small degree, and the ensuing process of neomorphosis tends to counteract the initial morphallaxis and restore the original form. Morgan[1] has shown that even two species of the same genus may differ materially in the ratio between remoulding of the old tissues and the formation of new, the latter process being more extensive in *P. lugubris* than in *P. maculata*. *Renilla* appears to offer a case in which both processes occur, but the former produces a result that is only temporary, owing to a high degree of specification in the members of the colony. That this specification is, however, not absolutely fixed is evident from the fact that heteromorphosis may occur, as shown in the formation of a new peduncle from a lateral group of polyps (Torrey), or in the development of a polyp instead of a peduncle from a severed axial polyp; and the same is proved by the establishment of a new colony after removal of the budding zone, as recorded above.

ZOÖLOGICAL LABORATORY OF COLUMBIA UNIVERSITY,
January 15, 1903.

[1] T. H. Morgan, "Growth and Regeneration in *Planaria lugubris*," *Arch. Entwm.*, XIII., 1, 2, 1901.

VARIATION NOTES.[1]

CARL H. EIGENMANN AND CLARENCE KENNEDY.

1. We have received twelve specimens of the cave salamander, *Spelerpes maculicaudus*, from Marble Cave, Mo., and other caves in the neighborhood. With one exception they presented the usual appearance of this species. They were red with dark spots bilaterally unsymmetrically scattered over the back and sides. The spots are subcircular and differ considerably in size. On the sides of the head and body and tail the spots not infrequently become confluent and give rise to short bars usually with their longer axes lengthwise of the specimens. Separating the dotted dorsal surface from the immaculate lower surface, there is an interrupted streak of dark much less intensely pigmented than the spots of the back. In a melanistic specimen from Rockhouse Cave, Mo., this lateral streak has become broad enough to cover the sides with a mottled pattern. The lower surface of the head is always more or less evenly peppered with isolated pigment cells.

The specimen to which attention is called is one exhibiting undoubted melanism. The lower surface of the head is more densely pigmented than in the other specimens. The sides are more uniformly pigmented than in the melanistic individual from Rockhouse. The sides of the head, body, the arms, and anterior surface of the legs are uniformly pigmented, except a few small blotches or spots. The pigmentation is not as intense as in the dorsal spots. The most striking deviation is found on the dorsal surface. The usual spots are present, rather smaller than in the other specimens. The intervening spaces are more densely covered with pigment cells than in the normal specimens and in several places, notably the head, the nape, and one or two places on the back the spots seem to have "*run*," their closely compacted pigment cells having been distributed in a thinner coat over a wider area and formed, with the similarly distributed pigment of other spots, diffuse, evenly pigmented blotches. In life

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 52.

the specimen suggested that the inhibitory force which kept these color cells from spreading, or the positive tropism which kept them together, was dissolved and the cells scattered evenly in a single layer over the surrounding region. The centers of distribution are still distinguishable as darker areas at the margin of or in the blotches. In the nape, for instance, four spots that were in part responsible for the blotch are seen at its four corners. It is very probable that color cells in addition to those originally forming the spots are concerned in forming the blotches.

2. A specimen of *Pygidium rivulatum*, 195 mm. long, a catfish from Lake Titicaca, has on the left side, in the place of the normal maxillary barbel, one which is dichotomously branched near its base into a dorsal and ventral branch. The dorsal branch is evidently the normal barbel, the ventral being the adventitious one. The ventral branch is but slightly shorter than the dorsal branch which is itself a little shorter than its fellow of the right side.

3. A specimen of *Xiphorhamphus jenynsii*, 155 mm.

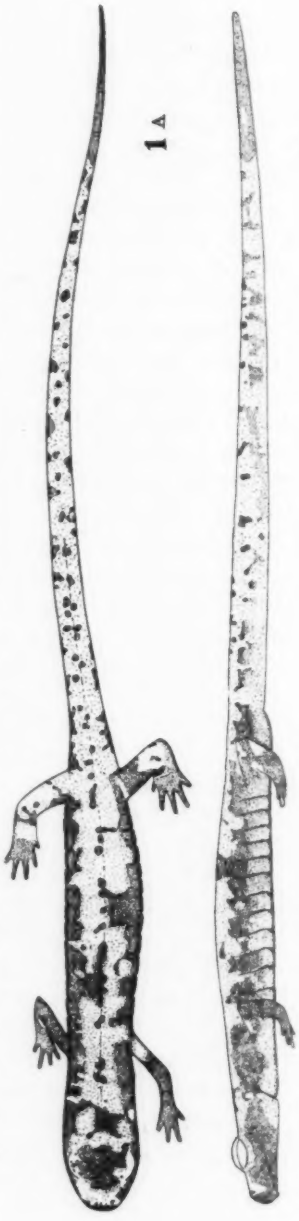

FIG. 1. *Spelerpes maculicaudus*.

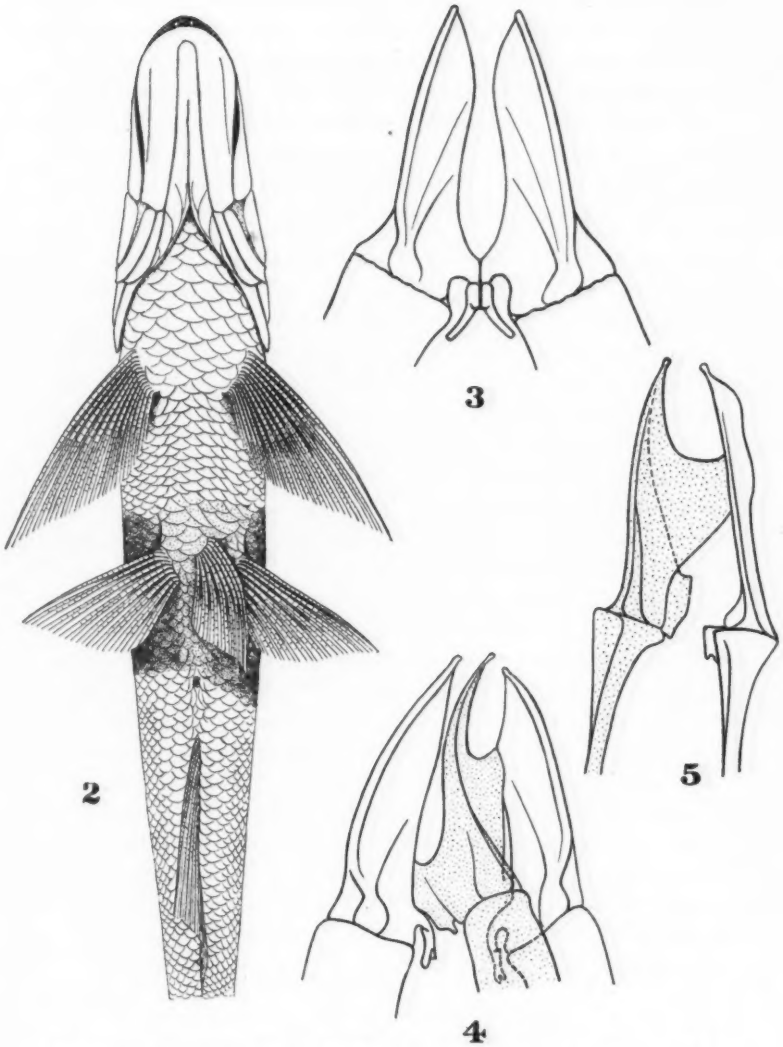

FIG. 2. Ventral surface of a *Xiphorhamphus jenynsii* with an adventitious ventral.
FIG. 3. The innominate bones of a normal *Xiphorhamphus*.
FIG. 4. The innominate bones of the specimen figured in 2.
FIG. 5. Left innominate and adventitious innominate from the left.

long, from the Rio Grande do Sul possesses an adventitious left ventral. The right and left ventrals are normally developed and of equal size. The left ventral is possibly placed a little higher than the right. The adventitious ventral is placed between the two normal ventrals slightly in advance of them and near the left one. It is shorter — 15 mm. as compared with 19 mm. — than the normal ventrals. It possesses one ray less — 8 instead of 9 — than the normal ones and its first ray is bent sickle-fashion.

THE CHROMOSOMES IN HEREDITY.

WALTER S. SUTTON.

In a recent announcement of some results of a critical study of the chromosomes in the various cell-generations of *Brachystola*[1] the author briefly called attention to a possible relation between the phenomena there described and certain conclusions first drawn from observations on plant hydrids by Gregor Mendel in[2] 1865, and recently confirmed by a number of able investigators. Further attention has already been called to the theoretical aspects of the subject in a brief communication by Professor E. B. Wilson.[3] The present paper is devoted to a more detailed discussion of these aspects, the speculative character of which may be justified by the attempt to indicate certain lines of work calculated to test the validity of the conclusions drawn. The general conceptions here advanced were evolved purely from cytological data, before the author had knowledge of the Mendelian principles, and are now presented as the contribution of a cytologist who can make no pretensions to complete familiarity with the results of experimental studies on heredity. As will appear hereafter, they completely satisfy the conditions in typical Mendelian cases, and it seems that many of the known deviations from the Mendelian type may be explained by easily conceivable variations from the normal chromosomic processes.

It has long been admitted that we must look to the organization of the germ-cells for the ultimate determination of hereditary phenomena. Mendel fully appreciated this fact and even instituted special experiments to determine the nature of that organization. From them he drew the brilliant conclusion that, while,

[1] Sutton, Walter S., "On the Morphology of the Chromosome Group in Brachystola magna," BIOL. BULL., IV., 1, 1902.

[2] Mendel, Gregor Johann, "Versuche über Pflanzen-Hybriden," *Verh. naturf. Vers. in Brünn* IV., and in Osterwald's *Klassiker der exakten Wissenschaft*. English translation in *Journ. Roy. Hort. Soc.*, XXVI., 1901. Later reprinted with modifications and corrections in Bateson's "Mendel's Principles of Heredity," Cambridge, 1902, p. 40.

[3] Wilson, E. B., "Mendel's Principles of Heredity and the Maturation of the Germ-Cells," *Science*, XVI., 416.

in the organism, maternal and paternal potentialities are present in the field of each character, *the germ-cells in respect to each character are pure.* Little was then known of the nature of cell-division, and Mendel attempted no comparisons in that direction; but to those who in recent years have revived and extended his results the probability of a relation between cell-organization and cell-division has repeatedly occurred. Bateson[1] clearly states his impression in this regard in the following words: "It is impossible to be presented with the fact that in Mendelian cases the cross-bred produces on an average *equal* numbers of gametes of each kind, that is to say, a symmetrical result, without suspecting that this fact must correspond with some symmetrical figure of distribution of the gametes in the cell divisions by which they are produced."

Nearly a year ago it became apparent to the author that the high degree of organization in the chromosome-group of the germ-cells as shown in *Brachystola* could scarcely be without definite significance in inheritance, for, as shown in the paper[2] already referred to, it had appeared that:

1. The chromosome group of the presynaptic germ-cells is made up of two equivalent chromosome-series, and that strong ground exists for the conclusion that one of these is paternal and the other maternal.

2. The process of synapsis (pseudo-reduction) consists in the union in pairs of the homologous members (*i. e.*, those that correspond in size) of the two series.[3]

3. The first post-synaptic or maturation mitosis is equational and hence results in no chromosomic differentiation.

4. The second post-synaptic division is a reducing division, resulting in the separation of the chromosomes which have conjugated in synapsis, and their relegation to different germ-cells.

5. The chromosomes retain a morphological individuality throughout the various cell-divisions.

[1] Bateson, W., "Mendel's Principles of Heredity," Cambridge, 1902, p. 30.

[2] Sutton, W. S., *loc. cit.*

[3] The conclusion that synapsis involves a union of paternal and maternal chromosomes in pairs was first reached by Montgomery in 1901.

Montgomery, T. H., Jr., "A Study of the Chromosomes of the Germ-Cells of Metazoa," *Trans. Amer. Phil. Soc.*, XX.

It is well known that in the eggs of many forms the maternal and paternal chromosome groups remain distinctly independent of each other for a considerable number of cleavage-mitoses, and with this fact in mind the author was at first inclined to conclude that in the reducing divisions all the maternal chromosomes must pass to one pole and all the paternal ones to the other, and that the germ-cells are thus divided into two categories which might be described as maternal and paternal respectively. But this conception, which is identical with that recently brought forward by Cannon,[1] was soon seen to be at variance with many well-known facts of breeding; thus:

1. If the germ-cells of hybrids are of pure descent, no amount of cross-breeding could accomplish more than the condition of a first-cross.

2. If any animal or plant has but two categories of germ-cells, there can be only four different combinations in the offspring of a single pair.

3. If either maternal or paternal chromosomes are entirely excluded from every ripe germ-cell, an individual cannot receive chromosomes (qualities) from more than one ancestor in each generation of each of the parental lines of descent, *e. g.*, could not inherit chromosomes (qualities) from both paternal or both maternal grandparents.

Moved by these considerations a more careful study was made of the whole division-process, including the positions of the chromosomes in the nucleus before division, the origin and formation of the spindle, the relative positions of the chromosomes and the diverging centrosomes, and the point of attachment of the spindle fibers to the chromosomes. The results gave no evidence in favor of parental purity of the gametic chromatin as a whole. On the contrary, many points were discovered which strongly indicate[2] that the position of the bivalent chromosomes

[1] Cannon, W. A., "A Cytological Basis for the Mendelian Laws," *Bull. Torrey Botanical Club*, 29, 1902.

[2] Absolute proof is impossible in a pure-bred form on account of the impossibility of distinguishing between maternal and paternal members of any synaptic pair. If, however, such hybrids as those obtained by Moenkhaus (Moenkhaus, W. J., "Early Development in Certain Hybrid Species," Report of Second Meeting of Naturalists at Chicago, *Science*, XIII., 323), with fishes can be reared to sexual maturity absolute proof of this point may be expected. This observer was able in the early cell[5]

in the equatorial plate of the reducing division is purely a matter of chance — that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the positions of other pairs — and hence that a large number of different combinations of maternal and paternal chromosomes are possible in the mature germ-products of an individual. To illustrate this, we may consider a form having eight chromosomes in the somatic and presynaptic germ-cells and consequently four in the ripe germ-products. The germ-cell series of the species in general may be designated by the letters A, B, C, D, and any cleavage nucleus may be considered as containing chromosomes *A*, *B*, *C*, *D* from the father and *a*, *b*, *c*, *d*, from the mother. Synapsis being the union of homologues would result in the formation of the bivalent chromosomes *Aa*, *Bb*, *Cc*, *Dd*, which would again be resolved into their components by the reducing division. Each of the ripe germ-cells arising from the reduction divisions must receive one member from each of the synaptic pairs, but there are sixteen possible combinations of maternal and paternal chromosomes that will form a complete series, to wit: *a*, *B*, *C*, *D*; *A*, *b*, *C*, *D*; *A*, *B*, *c*, *D*; *A*, *B*, *C*, *d*; *a*, *b*, *C*, *D*; *a*, *B*, *c*, *D*; *a*, *B*, *C*, *d*; *a*, *b*, *c*, *d*; and their conjugates *A*, *b*, *c*, *d*; *a*, *B*, *c*, *d*; *a*, *b*, *C*, *d*; *a*, *b*, *c*, *D*; *A*, *B*, *c*, *d*; *A*, *b*, *C*, *d*; *A*, *b*, *c*, *D*; *A*, *B*, *C*, *D*. Hence instead of two kinds of gametes an organism with four chromosomes in its reduced series may give rise to 16 different kinds; and the offspring of two unrelated individuals may present 16 × 16 or 256 combinations, instead of the four to which it would be limited by a hypothesis of parental purity of gametes. Few organisms, moreover, have so few as 8 chromosomes, and since each additional pair doubles the number of possible combinations in the germ-products[1] and quadruples that of the zygotes it is plain that in the ordinary form having from 24 to 36 chromosomes, the possibilities are immense. The table below shows the number of possible com-

of certain fish hybrids to distinguish the maternal from the paternal chromosomes by differences in form, and if the same can be done in the maturation-divisions the question of the distribution of chromosomes in reduction becomes a very simple matter of observation.

[1] The number of possible combinations in the germ-products of a single individual of any species is represented by the simple formula 2^n in which n represents the number of chromosomes in the reduced series.

binations in forms having from 2 to 36 chromosomes in the pre-synaptic cells.

Chromosomes.		Combinations in Gametes.	Combinations in Zygotes.
Somatic Series.	Reduced Series.		
2	1	2	4
4	2	4	16
6	3	8	64
8	4	16	256
10	5	32	1,024
12	6	64	4,096
14	7	128	16,384
16	8	256	65,536
18	9	512	262,144
20	10	1,024	1,048,576
22	11	2,048	4,194,304
24	12	4,096	16,777,216
26	13	8,192	67,108,864
28	14	16,384	268,435,456
30	15	32,768	1,073,741,824
32	16	65,536	4,294,967,296
34	17	131,072	17,179,869,184
36	18	262,144	68,719,476,736

Thus if Bardeleben's estimate of sixteen chromosomes for man (the lowest estimate that has been made) be correct, each individual is capable of producing 256 different kinds of germ-products with reference to their chromosome combinations, and the numbers of combinations possible in the offspring of a single pair is 256×256 or 65,536; while *Toxopneustes*, with 36 chromosomes, has a possibility of 262,144 and 68,719,476,736 different combinations in the gametes of a single individual and the zygotes of a pair respectively. It is this possibility of so great a number of combinations of maternal and paternal chromosomes in the gametes which serves to bring the chromosome-theory into final relation with the known facts of heredity; for Mendel himself followed out the actual combinations of two and three distinctive characters and found them to be inherited independently of one another and to present a great variety of combinations in the second generation.

The constant size-differences observed in the chromosomes of *Brachystola* early led me to the suspicion, which, however, a study of spermatogenesis alone could not confirm, that the individual chromosomes of the reduced series play different *rôles* in development. The confirmation of this surmise appeared later

in the results obtained by Boveri[1] in a study of larvæ actually lacking in certain chromosomes of the normal series, which seem to leave no alternative to the conclusion that the chromosomes differ qualitatively and as individuals represent distinct potentialities. Accepting this conclusion we should be able to find an exact correspondence between the behavior in inheritance of any chromosome and that of the characters associated with it in the organism.

In regard to the characters, Mendel found that, if a hybrid produced by crossing two individuals differing in a particular character be self-fertilized, the offspring, in most cases, conform to a perfectly definite rule as regards the differential character. Representing the character as seen in one of the original parents by the letter A and that of the other by a, then all the offspring arising by self-fertilization of the hybrid are represented from the standpoint of the given character by the formula AA : 2Aa : aa.—that is, one fourth receive only the character of one of the original pure-bred parents, one fourth only that of the other; while one half the number receive the characters of both original parents and hence present the condition of the hybrid from which they sprang.

We have not heretofore possessed graphic formulæ to express the combinations of chromosomes in similar breeding experiments, but it is clear from the data already given that such formulæ may now be constructed. The reduced chromosome series in *Brachystola* is made up of eleven members, no two of which are exactly of the same size. These I distinguished in my previous paper by the letters A, B, C, . . . K. In the unreduced series there are twenty-two elements[2] which can be seen to make up two series like that of the mature germ-cells, and hence may be designated as A, B, C . . . K + A, B, C . . . K. Synapsis results in the union of homologues and the production of a single series of double-elements thus: AA, BB, CC . . . KK, and the reducing division affects the separation of these pairs so that one member of each passes to each of the resulting germ-products.

[1] Boveri, Th., "Ueber Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verh. d. Phys.-Med. Ges. zu Würzburg*, N. F., Bd. XXXV., 1902. It appears from a personal letter that Boveri had noted the correspondence between chromosomic behavior as deducible from his experiments and the results on plant hybrids—as indicated also in footnote 1, *l. c.*, p. 81.

[2] Disregarding the accessory chromosome which takes no part in synapsis.

There is reason to believe that the division-products of a given chromosome in *Brachystola* maintain in their respective series the same size relation as did the parent element; and this, taken together with the evidence that the various chromosomes of the series represent distinctive potentialities, make it probable that a given size-relation is characteristic of the physical basis of a definite set of characters. But each chromosome of any reduced series in the species has a homologue in any other series, and from the above consideration it should follow that these homologues cover the same field in development. If this be the case chromosome *A* from the father and its homologue, chromosome *a*, from the mother in the presynaptic cells of the offspring may be regarded as the physical bases of the antagonistic unit-characters A and a of father and mother respectively. In synapsis, copulation of the homologues gives rise to the bivalent chromosome *Aa*, which as is indicated above would, in the reducing division, be separated into the components *A* and *a*. These would in all cases pass to different germ-products and hence in a monœcious form we should have four sorts of gametes,

$$A\,♂ \qquad a\,♂$$
$$A\,♀ \qquad a\,♀$$

which would yield four combinations,

$$A\,♂ + A\,♀ = AA$$
$$A\,♂ + a\,♀ = Aa$$
$$a\,♂ + A\,♀ = aA$$
$$a\,♂ + a\,♀ = aa$$

Since the second and third of these are alike the result would be expressed by the formula $AA : 2Aa : aa$ which is the same as that given for any character in a Mendelian case. *Thus the phenomena of germ-cell division and of heredity are seen to have the same essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same;* while as a corollary, it follows in each case that each of the two antagonistic units (chromosomes, characters) is contained by exactly half the gametes produced.

The observations which deal with characters have been made

chiefly upon hybrids, while the cytological data are the result of study of a pure-bred form; but the correlation of the two is justified by the observation of Cannon [1] that the maturation mitoses of fertile hybrids are normal. This being the case it is necessary to conclude, as Cannon has already pointed out, that the course of variations in hybrids either is a result of normal maturation processes or is entirely independent of the nature of those divisions. If we conclude from the evidence already given that the double basis of hybrid characters is to be found in the pairs of homologous chromosomes of the presynaptic germ-cells, then we must also conclude that in pure-bred forms likewise, the paired arrangement of the chromosomes indicates a dual basis for each character. In a hypothetical species breeding absolutely true, therefore, all the chromosomes or subdivisions of chromosomes representing any given character would have to be exactly alike, since the combination of any two of them would produce a uniform result. As a matter of fact, however, specific characters are not found to be constant quantities but vary within certain limits; and many of the variations are known to be inheritable. Hence it seems highly probable that homologous chromatin-entities are not usually of strictly uniform constitution, but present minor variations corresponding to the various expressions of the character they represent. In other words, it is probable that specific differences and individual variations are alike traceable to a common source, which is a difference in the constitution of homologous chromatin-entities. Slight differences in homologues would mean corresponding, slight variations in the character concerned — a correspondence which is actually seen in cases of inbreeding, where variation is well known to be minimized and where obviously in the case of many of the chromosome pairs both members must be derived from the same chromosome of a recent common ancestor and hence be practically identical.

In the various forms of parthenogenesis we meet the closest kind of inbreeding and a brief consideration of the variability to be expected in each, from the standpoint of the chromosome theory, may serve as a guide to such research as will test the

[1] Cannon, W. A., *loc. cit.*

validity of the latter. The simplest form, of which chemical parthenogenesis in sea-urchins is an example, is that in which the organism has only a single chromosome series, to be represented by *A*, *B*, *C*, *D*...*N*. Thus far no recognized cases of this type have been reared to sexual maturity, but it is to be expected that no reducing division will be found in the maturation of such forms, and that their parthenogenetic offspring will exactly resemble the immediate parent.

In cases of natural parthenogenesis which are accompanied by the reëntrance of the second polar body and its fusion with the egg-nucleus (or its failure to form) there must be a double chromosome series; but we may distinguish two classes according as the reducing process is accomplished in the first or the second maturation division.[1] If reduction is accomplished in the first division, one half the chromosomes of the oögonia are thrown out and lost in the first polar body. The second division, being equational, would result in a polar body which would be the exact duplicate of the egg-nucleus as far as chromosomes are concerned and which accordingly, by its reëntrance would add nothing new to the egg-series. The series after fusion would, therefore, be represented by the letters *A*, *B*, *C*, *D*...*N* + *A*, *B*, *C*, *D*...*N*. If such a type of parthenogenesis were to follow sexual reproduction, the first generation of offspring might be expected to differ materially from the parent by reason of the casting out, in the first polar body, of chromosomes representing certain dominant characters, and the consequent appearance in the offspring of the corresponding recessives. Subsequent parthenogenetic generations, however, would in each case be endowed with a chromosome series exactly similar to that of the immediate parent and accordingly might be expected to show the same characters.

In case the second division of a parthenogenetic egg were the reducing division, the reëntrance or suppression of the second polar body would accomplish the restoration of the oögonial chromosome-series. In this case the first parthenogenetic gen-

[1] Either must be regarded as possible in cases where we have no definite knowledge since it is regularly described as the second in the Orthoptera (McClung, Sutton and Copepoda (Rückert, Häcker) while in the Hemiptera-Heteroptera it is believed to be the first (Paulmier, Montgomery).

eration might be expected to duplicate the characters of the parent (if environmental conditions remained unchanged) and little or no variability would be expected as long as parthenogenesis persisted.

In relation to these problems there is great need of a simultaneous study of the germ-cell divisions and the variation of periodically parthenogenetic forms.

We have seen reason, in the foregoing considerations, to believe that there is a definite relation between chromosomes and allelomorphs[1] or unit characters but we have not before inquired whether an entire chromosome or only a part of one is to be regarded as the basis of a single allelomorph. The answer must unquestionably be in favor of the latter possibility, for otherwise the number of distinct characters possessed by an individual could not exceed the number of chromosomes in the germ-products; which is undoubtedly contrary to fact. We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together. On the other hand, it is not necessary to assume that all must be apparent in the organism, for here the question of dominance enters and it is not yet known that dominance is a function of an entire chromosome. It is conceivable that the chromosome may be divisible into smaller entities (somewhat as Weismann assumes), which represent the allelomorphs and may be dominant or recessive independently. In this way the same chromosome might at one time represent both dominant and recessive allelomorphs.

Such a conception infinitely increases the number of possible combinations of characters *as actually seen* in the individuals and unfortunately at the same time increases the difficulty of determining what characters are inherited together, since usually recessive chromatin entities (allelomorphs?) constantly associated in the same chromosome with usually dominant ones would evade detection for generations and then becoming dominant might appear as reversions in a very confusing manner.

[1] Bateson's term.

In their experiments on *Matthiola*, Bateson and Saunders[1] mention two cases of correlated qualities which may be explained by the association of their physical bases in the same chromosome. "In certain combinations there was close correlation between (*a*) green color of seed and hoariness, (*b*) brown color of seed and grabrousness. In other combinations such correlation was entirely wanting." Such results may be due to the association in the same chromosomes of the physical bases of the two characters. When close correlation was observed, both may be supposed to have dominated their homologues; when correlation was wanting, one may have been dominant and the other recessive. In the next paragraph to that quoted is the statement: "The rule that plants with flowers either purple or claret arose from green seeds was universal." Here may be a case of constant dominance of two associated chromatin-entities.

Dominance is not a conception which grows out of purely cytological consideration. Cytology merely shows us the presence in a cell of two chromosomes, either of which is capable of producing some expression of a given character, and it is left to experiment in each case to show what the effect of this combined action will be. The experiment[2] has shown that any one of the three theoretical possibilities may be realized, viz: (1) One or the other may dominate and obscure its homologue. (2) The result may be a compromise in which the effect of each chromosome is to be recognized. (3) The combined action of the two may result in an entirely new cast of character. In cases belonging to the first category, the visible quality (allelomorph, chromatin-entity) was described by Mendel as dominant and the other as recessive, and the experiments of Bateson and Saunders and others, as well as those of Mendel, have shown that in many cases a dominant character tends to remain dominant during successive generations if the environment is not materially changed. Nevertheless, some experiments cited by Bateson[2] go to show that dominance may be variable or defective. Furthermore, it is not only conceivable, but highly probable that in most, if not all

[1] Bateson and Saunders, Experimental Studies in the Physiology of Heredity. Reports to the Evolution Committee, I. London, 1902, p. 81, paragraphs 11 and 12.

[2] *Cf.* Bateson and Saunders, *loc. cit.*

cases, there are many different expressions of each character (*i. e.*, many different allelomorphs as suggested by Bateson[3] in regard to human stature), which on various combinations would necessarily exhibit relative dominance. The experiments with peas show an almost constant dominance of certain allelomorphs, such as round over wrinkled in seeds, and of yellow over green in cotyledons; but it is worthy of note that here, as in most Mendelian experiments, only two antagonistic characters have been used. Investigations on varieties, in general similar, but exhibiting different expressions of some particular character, will certainly yield instructive results. Bateson's observations on crosses between single-, rose- and pea-combed fowls, represent a simple form of such a case and may be expected on completion to add much to our knowlege of the nature of dominance.

In addition to the many examples brought forward by Bateson in support of the Mendelian principle he cites three types of cases which are to be regarded as non-Mendelian. These are:

1. The ordinary blended inheritance of continuous variation.
2. Cases in which the form resulting from the first cross breeds true.
3. The "false hybrids" of Millardet.

1. *Blended Inheritance.* — In treating of this class Bateson clearly states the possibility that the case may be one entirely "apart from those to which Mendel's principles apply," but goes on to show how it may possibly be brought into relation with true Mendelian cases. He says in part: "It must be recognized that in, for example, the stature of a civilized race of man, a typically continuous character, there must certainly be on any hypothesis more than one pair of possible allelomorphs. There may be many such pairs, but we have no certainty that the number of such pairs and consequently of the different kinds of gametes are altogether *unlimited*, even in regard to stature. If there were even so few as, say, four or five pairs of possible allelomorphs, the various homo- and heterozygous combinations might, on seriation, give so near an approach to a continuous curve that the purity of the elements would be unsuspected, and their detection practically impossible." This hypothesis, which presents no difficulties from the point of view of the chromosome theory, is

sufficient in the present state of our knowledge to bring many cases of apparently continuous variation into definite relation with strictly Mendelian cases; but, on the other hand, it seems probable, as already noted (p. 221), that the individual variation in many characters now thought to be strictly Mendelian may prove to be due to the existence in the species of many variations of what may be regarded as the type allelomorphs, accompanying similar variations of the homologous chromatin entities representing those types.

2. *First Crosses that Breed True.* — It is obvious that in the germ-cells of true-breeding hybrids[1] there can be no qualitative reduction. In the normal process synapsis must be accounted for by the assumption of an affinity existing between maternal and paternal homologues, and conversely reduction is the disappearance of that affinity or its neutralization by some greater force. Now in *Hieracium* the characters of the hybrid are frequently intermediate between those of the two parents, showing that both allelomorphs (or chromatin-entities) are at work, but on self fertilization there is no resolution of allelomorphs (reduction division). On the contrary, all the germ-cells are equivalent, as shown by the fact that all combinations produce similar offspring which in turn are similar to the parent. The suggestion made by Bateson in another connection, that "if one allelomorph were alone produced by the male and the other by the female we should have a species consisting *only* of heterozygotes," which would come true as long as bred together, at first sight seems logically applicable to these cases. For such an idea, however, we can find no cytological justification, since if any reduction occurs both chromosomes occur in both male and female germ-cells in equal numbers; and further, the evidence is in favor of a great variety of combinations of maternal and paternal chromosomes in the germ-cells so that the exact chromosome group of a hybrid parent could hardly be duplicated except by fusion of the very pair of cells separated by the reducing division. A more plausible explanation from the cytological standpoint is that the union of the chromosomes in synapsis is so firm that no reduction can take place, *i. e.*, that in each case, a paternal and

[1] *Cf.* Mendel's experiments on *Hieracium*.

a maternal chromosome fuse permanently to form a new chromosome which subsequently divides only equationally. The result must be germ-cells which are identical with one another and with those of the parents, and hence self-fertilization would produce offspring practically without variation. If this explanation be the correct one the process is distinctly pathological and hence it is not surprising that such cases, as noted by Bateson, should often present "a considerable degree of sterility."

3. *The "False Hybrids" of Millardet.* — Millardet, de Vries and Bateson have all described experiments in which the offspring resulting from a cross between dissimilar individuals showed the character of one parent only, those of the other parent being shown by further experiment to be lost permanently. The obvious cytological explanation of such a phenomenon is hinted at by Bateson in the words "Such phenomena may perhaps be regarded as fulfilling the conception of Strasburger and Boveri, that fertilization may consist of two distinct operations, the stimulus to development and the union of characters in the zygote."[1] Division of the egg without fusion of the pronuclei in a well-known phenomenon having been observed in eggs treated with chloral (Hertwig brothers) or ether (Wilson) and may be supposed to occur under certain unusual conditions in nature. In the experiments mentioned, however, both pronuclei continue to divide separately, while for a cytological explanation of the occurrence of "false hybrids" it is necessary to conceive not only the failure of the nuclei to copulate but the entire disappearance of one of them. Such a case would be comparable to that of chemically induced parthenogenesis or to the fertilization of enucleate egg-fragments, according as the nucleus remaining was maternal or paternal. Speculation in this connection, however, is unprofitable excepting so far as it may serve as a guide to research. A careful study of the fertilization of such cases as Millardet's strawberries, de Vries's *Oenothera* and Bateson's *Matthiola* crosses will no doubt be productive of immediate and positive results.

Mosaics.— A fourth class of non-Mendelian cases, the "mosaics" or "piebalds" constitute a group in relation to which, as I believe, only negative evidence is to be expected

[1] Bateson and Saunders, *loc. cit.*, p. 154.

from direct cytological study. A good example of the class is the "mosaic" fruit of *Datura* obtained by Bateson and Saunders, which, although in general exhibiting the thornless recessive condition, showed in exceptional cases a thorny patch. Of this case Bateson says: "Unless this is an original sport on the part of the individual, such a phenomenon may be taken as indicating that the germ-cells may also have been mosaic." I must confess my failure to comprehend just what is here meant by mosaic germ-cells. I have attempted to show that in all probability the germ-cells are normally a mosaic of maternal and paternal chromosomes, but very evidently this is not Bateson's meaning.

From the standpoint of the chromosome theory I would suggest a possible explanation of the conditions as follows: We have already assumed that the somatic chromosome group, having a similar number of members to that of the cleavage nucleus and derived from it by equation divisions, is made up in the same way of pairs of homologous chromosomes. Every somatic cell, by this conception, must contain a double basis in the field of each character it is capable of expressing. In strictly Mendelian cases one of the homologues is uniformly dominant throughout the parts of the organism in which the character is exhibited. As already noted, however, it is unlikely that all the descendants of a dominant chromatin entity will be dominant. This is shown by the experiment of de Vries with sugar beets, which are normally biennial but always produce a small percentage of annual plants or "runners," which latter are regarded as recessives. The percentage of these runners may be increased by rearing the plants under unfavorable conditions and this is taken as evidence that the recessive allelomorphs may become dominant under such conditions.[1]

If each cell contains maternal and paternal potentialities in regard to each character, and if dominance is not a common function of one of these, there is nothing to show why as a result of some disturbing factor one body of chromatin may not be called into activity in one group of cells and its homologue in another. This would produce just the sort of a mosaic which Bateson and

[1] *Cf.* Bateson and Saunders, pp. 135, 136.

Saunders found in *Datura* or as Tchermak's pied yellow and green peas obtained by crossing the *Telephone* pea with yellow varieties. Correns describes the condition as *pœcilodynamous* and his conception of the causes of the phenomenon as I understand it is parallel with that which I have outlined above. The logical possibility suggested by Bateson[1] that the recessive islands in such cases as the mosaic pea may be due to recessive allelomorphs in the paired state does not accord with the theory of a chromosomic basis for those allelomorphs, since the chromosome groups, both of cells showing the recessive character and of neighboring cells showing the dominant one, are derived, so far as we know, by longitudinal or equation division from the chromosomes of the same original cleavage nucleus and hence must be alike.

The application of the theory here suggested may be put to test by an experiment in which hybrids of dissimilar true-breeding parentage are crossed and a third generation of "quarter-bloods" produced. Mosaics occurring in such an organism, if this theory be correct, would show one character resembling that of one of the maternal grandparents and one resembling that of one of the original pure-breds of the paternal side. If both characters of the mosaic should be clearly paternal or maternal the theory as outlined is proven inadequate, since one of each pair of chromosomes, and hence the corresponding character-group, is thrown out by the reduction-division in each generation.

In considering the behavior of the two chromosomes forming the basis of any given character, it was noted that in some cases the heterozygote character resulting from the combinations of dissimilar allelomorphs is sometimes totally unlike either of the latter. Thus Mendel found that in crosses between peas respectively 1 and 6 feet in height the offspring ranged from 6 to 7½ feet. In discussing similar cases, Bateson calls attention to the light which would be thrown on the phenomenon if we ventured to assume that the bases of the two allelomorphs concerned are chemical compounds; and he compares the behavior of the allelomorphs to the reaction of sodium and chlorine in the formation

[1] Bateson and Saunders, p. 156.

of salt. The results of chemical analysis show that one of the most characteristic features of chromatin is a large percentage content of highly complex and variable chemical compounds, the nucleo-proteids, and therefore if, as assumed in the theory here advanced, the chromosomes are the bases of definite hereditary characters, the suggestion of Bateson becomes more than a merely interesting comparison.

We have seen reason in the case of the true-breeding hybrids to suspect that the transmission by the hybrid of heterozygote characters may be due to permanant union of the homologous chromosomes. From this it is but a short step to the conclusion that even if, as is normally the case, the chromosomes do not fuse permanently, the very fact of their association in the same liquid medium may allow a possibility of a certain degree of chemical interaction. This must normally be slight, since its effects do not appear to be visible in a single generation; but the slightest of variation as a result of repeated new association, even though it tend in diverse directions, must in time, guided by natural selection, result in an appreciable difference in a definite direction between a chromosome and its direct descendant and hence between the characters associated with them. In this we have a suggestion of a possible cause of individual variation in homologous chromosomes which we have already seen reason to suspect (pp. 221 and 226).

Finally, we may briefly consider certain observations which seem at first sight to preclude the general applicability of the conclusions here brought out. If it be admitted that the phenomenon of character-reduction discovered by Mendel is the expression of chromosome-reduction, it follows that forms which vary according to Mendel's law must present a reducing division. But the vertebrates and flowering plants—the very forms from which most of the Mendelian results have been obtained—have been repeatedly described as not exhibiting a reducing division. Here, therefore, is a discrepancy of which I venture to indicate a possible explanation in the suggestion first made by Fick[1] and more recently by Montgomery.[2] This is to the effect that in

[1] Fick, R., "Mittheilung ueber Eireifung bei Amphibien," *Supp. Anat. Anz.*, XVI.
[2] Montgomery, T. H., Jr., *loc. cit.*

synapsis as it occurs in vertebrates and other forms possessing loop-shaped chromosomes, the union is side by side instead of end-to-end ~~to~~ as in Arthropods. In vertebrates, two parallel longitudinal splits, the forerunners of the two following divisions, appear in the chromosomes of the primary spermatocyte prophases. Both being longitudinal, they have been described as equation divisions, but if it shall be found possible to trace one to the original line of union of the two spermatogonial chromosomes side by side in synapsis, that division must be conceived as a true reduction. A number of observations supporting this view will be brought forward in my forthcoming work on *Brachystola*.

Again, if the normal course of inheritance depends upon the accurate chromatin-division accomplished by mitosis, it would appear that the interjection, into any part of the germ cycle, of the gross processes of amitosis could result only in a radical deviation from that normal course. Such an occurrence has actually been described by Meves, McGregor and others in the primary spermatogonia of amphibians. In these cases, however, it appears that fission of the cell-body does not necessarily follow amitotic division of the nucleus. I would suggest, therefore, the possibility that the process may be of no significance in inheritance, since by the disappearance of the nuclear membranes in preparation for the first mitotic division, the original condition is restored, and the chromosomes may enter the equatorial plate as if no amitotic process had intervened.

There is one observation in connection with the accessory chromosome which deserves mention in any treatment of the chromosomes as agents in heredity. This element always divides longitudinally and hence probably equationally. It fails to divide in the first maturation mitosis, in which the ordinary chromosomes are divided equationally, but passes entire to one of the resulting cells. In the second maturation division, by which the reduction of the ordinary chromosomes is effected, the accessory divides longitudinally.[2]

[1] It is of interest in connection with this question that there occurs regularly in each of the spermatogonial generations in *Brachystola* a condition of the nucleus which suggests amitosis but which in reality is nothing more than the enclosure of the different chromosomes in partially separated vesicles. Cf. Sutton, W. S., "The Spermatogonial Divisions in Brachytola Magna," *Kans. Univ. Quart.*, IX., 2.

[2] The chromosome *x* of *Protenor*, which of all chromosomes in non-orthopteran

My observations in regard to the accessory chromosome lend support to the hypothesis of McClung[1] that of the four spermatozoa arising from a single primary spermatocyte, those two which contain this element enter into the formation of male offspring, while the other two, which receive only ordinary chromosomes take part in the production of females. If this hypothesis be true, then it is plain that in the character of sex the reduction occurs in the first maturation mitosis, since it is this division which separates cells capable of producing only males from those capable of producing only females. Thus we are confronted with the probability that reduction in the field of one character occurs in one of the maturation divisions and that of all the remaining characters in the other division. The significance of such an arrangement, though not easy of perception, is nevertheless great. As regards their chromosome groups, the two cells resulting from each reduction mitosis are conjugates and, therefore, opposites from the standpoint of any individual character. Thus if we consider a hypothetical form having eight chromosomes comprising the paternal series *A*, *B*, *C*, *D* and the maternal series *a*, *b*, *c*, *d*, one of the cells resulting from the reduction division might contain the series *A*, *b*, *c*, *D*, in which case its sister-cell would receive the conjugate series *a*, *B*, *C*, *d*. It is plain that these conjugates, differing from each other in every possible character, represent the most widely different sperms the organism can produce. Now if reduction in the sex-determining chromatin also took place in this division it is apparent that these two diametrically opposite series would enter into individuals of different sexes; but if the sex-reduction is previously accomplished by the asymmetrical distribution of the accessory in the first division, then both the members of each conjugate pair must take part in the production either of males or of females and thus

forms most closely resemble the accessory, is also described by Montgomery (1901) as dividing in the reducing division, and failing to divide in the equation division—a fact which is the more remarkable because in *Protenor*, as in all Hemiptera-Heteroptera thus far described, reduction is accomplished in the *first* maturation division.

[1] McClung, C. E., "The Accessory Chromosome — Sex Determinant?" BIOL. BULL., III., 1 and 2, 1902. "Notes on the Accessory Chromosome," *Anat. Anz.*, XX., pp. 220–226.

all extremes of chromosome combination are provided for within the limits of each sex.

Postscript.

The interesting and important communication of Guyer[1] on "Hybridism and the Germ-Cell" is received too late for consideration in the body of this paper. This investigator also has applied conclusions from cytological data to the explanation of certain phenomena of heredity, and his comparative observations on the spermatogenesis of fertile and infertile hybrids are an important contribution to the cytological study of the subject. The conclusions drawn are of great interest but, I think, in some cases, open to criticism. In assuming that there is a "segregation of maternal and paternal chromosomes into separate cells, which may be considered 'pure' germ-cells containing qualities of only one species" (p. 19), he repeats the error of Cannon which has already been dealt with in the early part of this paper. No mention is made in the paper of Mendel's law but in considering the inbred pigeon hybrids from which his material was obtained, the author expresses his familiarity with manifestations of the Mendelian principle by the statement that "in the third generation there is generally a return to the original colors of the grandparents." In cases which seem to resemble one grandparent in all particulars it is clear that the conception of pure germ-cells may be strictly applied, but the author was familiar with cases of inbred hybrids which plainly show mixtures. These he is inclined to explain in two ways as follows: (1) "Union of two cells representing each of the two original species would yield an offspring of the mixed type." (2) "Besides through the mixing just indicated, variability may be due also in some cases to the not infrequent inequalities in the division of individual chromosomes, through which varying proportions of the chromatin of each species may appear in certain of the mature germ-cells" (p. 20).

The first of these explanations would accord with the result of Mendelian experiment but for the fact that it is erroneously applied (and without cytological grounds) to *all* the characters or chromosomes instead of to individuals. As for the second

[1] Guyer, M. F., "Hybridism and the Germ-Cell," *Bulletin of the University of Cincinnati*, No. 21, 1902.

passage quoted, there can be little doubt that irregular division of chromosomes would be likely to produce marked variation, but as Guyer himself observes, *these irregularities increase with the degree of infertility.* It seems natural to conclude, therefore, that they are not only pathological but perhaps in part the cause of the infertile condition. Furthermore, on the hypothesis of individuality of chromosomes, which Guyer accepts, the loss of a portion of a chromosome by irregular division would be permanent and the effect of repetitions of the operation upon the descendants of a single chromosome group (which he regards as transmitted as a whole) would be so marked a depletion of chromatic substance as must lead soon to malfunction and ultimately to sterility.

As already noted (p. 216) the first of these two explanations of the causes of variation would allow only four possible combinations of chromosomes in the offspring of a single pair. But we know that except in the case of identical twins, duplicates practically never appear in the offspring of a pair however numerous the progeny. Therefore, whatever the number of the offspring, the variations of all except the few provided for by the four normal chromosome combinations must be accounted for by obviously pathological division processes, which tend strongly in the direction of sterility. But in the report of Bateson and Saunders to the Evolution Committee we find the statement: "We know no Mendelian case in which fertility is impaired" (p. 148). When we reflect that the vast majority of cases studied by these observers were Mendelian and connect this piece of evidence with the testimony of Cannon[1] that the maturation processes of variable cotton-hybrids are either normal or so distinctly abnormal as to entail sterility and with Guyer's own admission that the abnormalities in mitosis increase with the degree of sterility, the balance is strongly against the efficacy of pathological mitoses as factors in normal hybrid variation.

I take pleasure in acknowledging my indebtedness to Professor E. B. Wilson for invaluable counsel in the presentation of a subject offering many difficulties.

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY,
January 25, 1903.

[1] Cannon, W. A., *loc. cit.*

ON PHYLLODISTOMUM AMERICANUM (N. SP.); A NEW BLADDER DISTOME FROM AMBLYSTOMA PUNCTATUM.

HENRY LESLIE OSBORN.

PART I.

The species of this genus which have been reported hitherto have all come from the old world. *P. folium* Olf., from Europe,

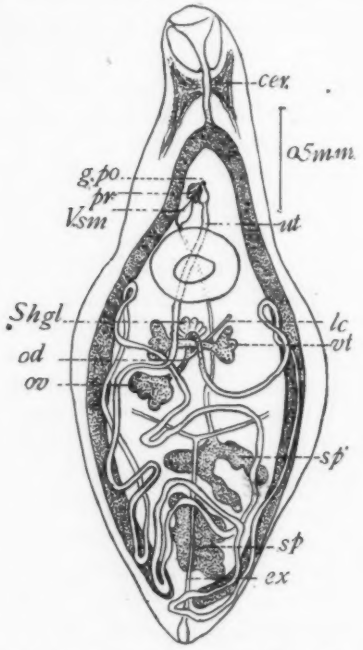

FIG. 1. Ventral view of *P. americanum*, camera lucida, × 32 diam. *g.po.*, genital pore; *ut.*, uterus; *l. c.*, Laurer's canal; *vt.*, vitellaria; *V. def.*, vas deferens; *sp.'* anterior testis; *sp.''*, posterior testis; *ex.*, excretory bladder; *int.*, intestine; *ov.*, ovary; *Sh.gl.*, shell gland; *V.sm.*, seminal vesicle; *pr.*, prostate cells.

and *P. patellare*, Sturgis, '97, from Japan, have been till recently the only species known; during 1902, however, Odhner added

four species from fishes of northeastern Africa. I have been greatly interested to find that some of the salamanders of this neighborhood, *Amblystoma tigrinum*, are infected with a species of *Phyllodistomum* unlike any hitherto reported. Pending a later fuller account of the structure of the fluke, this brief notice will indicate the chief points in its anatomy.

The worm is rather uncommon. In twenty-nine salamanders that have been examined for it it has been found in only six cases.

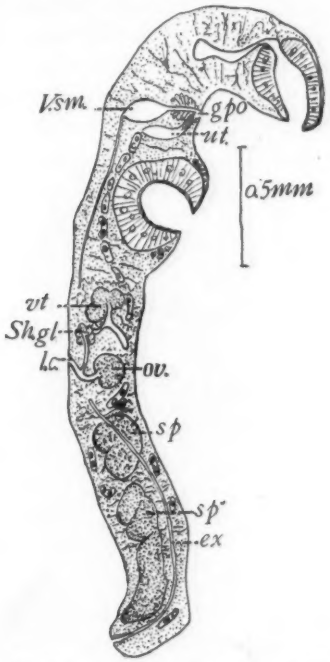

FIG. 2. Sagittal section, × 36. Camera lucida; lettering as in Fig. 1.

The parasite is found in the urinary bladder of the salamander. In one case nineteen flukes were found, but this was exceptional, for two, three or four mature flukes is the maximum found in the other cases, beside perhaps two or three small flukes apparently a young stage of the others. The coarser features of the organization are shown in Fig. 1, a ventral view from a specimen preserved (without compression) in corrosive sublimate solution and stained,

cleared and mounted whole. The form of the body is less spatulate than that of *P. folium* or *P. patellare*, the neck is less distinct. The total length of this specimen is 3.5 mm., its greatest breadth is 1.4 mm., the ratio of breadth to length is thus 40 per cent. In *P. patellare* this ratio is 66 per cent., in *P. spatula* (Odhner, '02) it is 48 per cent. The American form is thus less broad than any reported old world form. There is considerable variation in this respect in my material, some cases being decidedly slender, in one mature specimen studied alive under some compression and measured from the camera lucida drawing, the length is 4.2 mm., the width 0.88 and the ratio of breadth to length 20.9 per cent. This specimen is more than usually narrow, most having the broadened form of Fig. 1.

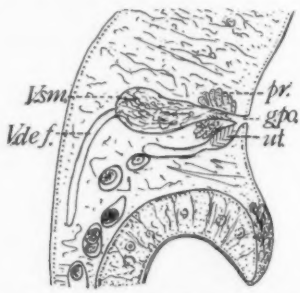

FIG. 3. Section at the genital pore, × about 120.

There is no pharynx, the œsophagus is short, the intestines branch well forward, and are very long, reaching to the extreme posterior end of the body. The cells of the intestine are provided with very long cilia, as in *P. patellare;* they would probably be very interesting subjects for histological study. In both *P. folium* and *P. patellare* the intestines are somewhat sacculated, but here they are entirely simple.

The excretory system is like that of *P. folium* and *P. patellare* — dorsal posterior terminal pore, a long ventrally located bladder branching anteriorly on the level of the hinder boundary of the ovary. Smaller vessels and flame cells like Fig. 5 of Sturgis, '97, are recognizable in sections.

The chief important internal differences between this species and those previously known are in the reproductive system. The

two testes are both nearly in the middle line, the anterior slightly more on the left side, both are entirely within the hinder third of the body. The anterior testis lies wholly posterior to the ovary, unlike either *P. folium* where it lies on the same level as the ovary, or *P. patellare* where a considerable part is anterior to the ovary. Both testes are very deeply and irregularly lobed, but they are not divided. The lobing is deeper than in any other species of the genus, in some of which (*e. g.*, *P. unicum*, Odhner, '02) they are entire. The cirrhus organ is present, not enveloped in a sac, there is a small seminal vesicle, the ductus ejaculatorius is ciliated and surrounded by prostate cells not marked off from the parenchyma by a membrane. The genital pore is situated in the middle line some distance in front of the ventral sucker.

The ovary is located on the right side and in front of the anterior testis (usually, but on the left side in occasional in-

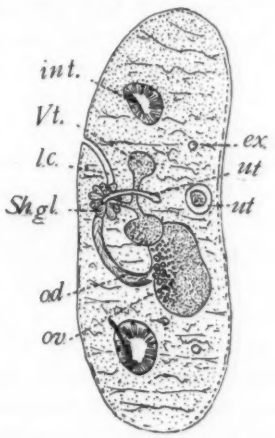

FIG. 4. Combined view from several serial sections showing the relation of the ovary and the neighboring parts, all represented as if on the same level, × about 120.

stances). It is deeply lobed. The oviduct arises from its dorsal surface (see Fig. 4) and passes toward the anterior end. A passage (Laurer's canal) is given off from the oviduct and runs to the dorsal surface and right side and there opens to the exterior. There is no seminal receptacle. The oviduct running on passes through a distinct shell gland, receiving at this place also the duct from the vitellaria; it then passes on as the uterus arching

over the vitelline duct and passing backward toward the posterior end of the body. The course of the uterus is unlike that of any described member of this genus, and is shown in detail in Fig. 1, where however the coils are somewhat simplified for the sake of clearness. The course is first a forward loop on the left side, then on the same side a loop running to the hind end of the body, having on its return part a side loop down into the space between the two testes, then another loop across the front of the anterior testis, then the uterus crosses to the right side and forms first an anterior loop and finally a long posterior one, at last passing across in front of the ovary and ventrally to and between the two vitella and then dorsally over the ventral sucker to reach the genital pore. There is hardly any atrium (Fig. 3), the male and female ducts meeting almost as they reach the surface. The terminal part of the uterus is ciliated like that of the ductus ejaculatorius. The ova measure 0.052 mm. x 0.050 mm.

The vitellaria lie close together near the center of the body. They are lobed, each consisting of about three parts, one in the center and one in front of this and one behind it. These are not separate follicles, but lobes of a single organ.

Of the generic identity of this form with *Phyllodistomum* of Braun, '99, there can be no doubt. The specific distinctness is equally clear. As it is the first form to be reported from this country I propose the name *americanum* to designate the species.

Part II.

Since the foregoing part of this article was written an article has been published by Stafford, '02, on the American Representatives of *Distomum cygnoides*, in which a new form is described under the name of *Gorgodera translucida*, which bears a considerable resemblance to *P. americanum*. I have also had an opportunity which I owe to the kindness of Professor Stafford of examining some specimens of the species he has described.

The transparency so noticeable in Stafford's form is also noticeable in some of my specimens. My material is derived from two sources, first from worms found by an assistant (Mr. C. C. Tyrrell) in the laboratory and at once (except one studied alive) placed in corrosive sublimate solution, which are all opaque, and

specimens found by opening the bladders of salamanders which had been preserved in formaline for anatomical work. These latter were, many of them, quite decidedly translucent. I think it likely that as soon as I can obtain living specimens I shall find them at least somewhat translucent if less so than Stafford's.

The form of the body in *G. translucida* is slender and parallel-sided, and not at all inclined to a spatulate form. While this is quite unlike the form of Fig. 1, there are among my specimens some in which the body is very slender. I have not yet decided to what extent this may be due to the youth of the specimen. Young worms found in bladders with mature specimens and apparently earlier forms of this species are decidedly slender in outline and not spatulate. I have even found some in which eggs were already filling the uterus in which this elongate form was still psesent. I have little doubt that fully mature older specimens all become spatulate eventually. These facts indicate a gradual shading from one to the other of these contrasted body forms.

There appears also to be considerable similarity in the arrangement of the coils of the uterus in these two forms.

The location of the ventral sucker is much farther forward in *G. translucida* than in *P. americanum*, being in the former 20 per cent. of the total length from the anterior end and in the latter 32–42 per cent. The testes are located in the hinder third of the body and are deeply lobed in *P. americanum*, and are in the middle third and are nearly entire in *G. translucida*. The ovary is behind the middle of the body not near the ventral sucker and is deeply lobed in *P. americanum*, and is in front of the middle of the body near the ventral sucker and entire in *G. translucida*.

These differences are not entitled to be rated as of sufficient value to justify placing these forms in different genera, unless the body form proves to be a difference of more importance than at present appears. It is the only character that is offered by Looss, '99, by which to distinguish his *Spathidium* (= *Phyllodistomum* of Braun, '99) from *Gorgodera*, though some species of the latter (*e. g.*, *G. cygnoides*) differ in having many testes instead of a single pair. For the present and until more is known

of *P. americanum* the spatulate form will have to remain the distinguishing mark of the genus. Should its rank as a critical feature be lost, it seems that we should then be obliged to bring *Gorgodera translucida*, and with it some other species of the genus into the genus *Phyllodistomum*.

HAMLINE UNIVERSITY, ST. PAUL, MINN.,
February 4, 1903.

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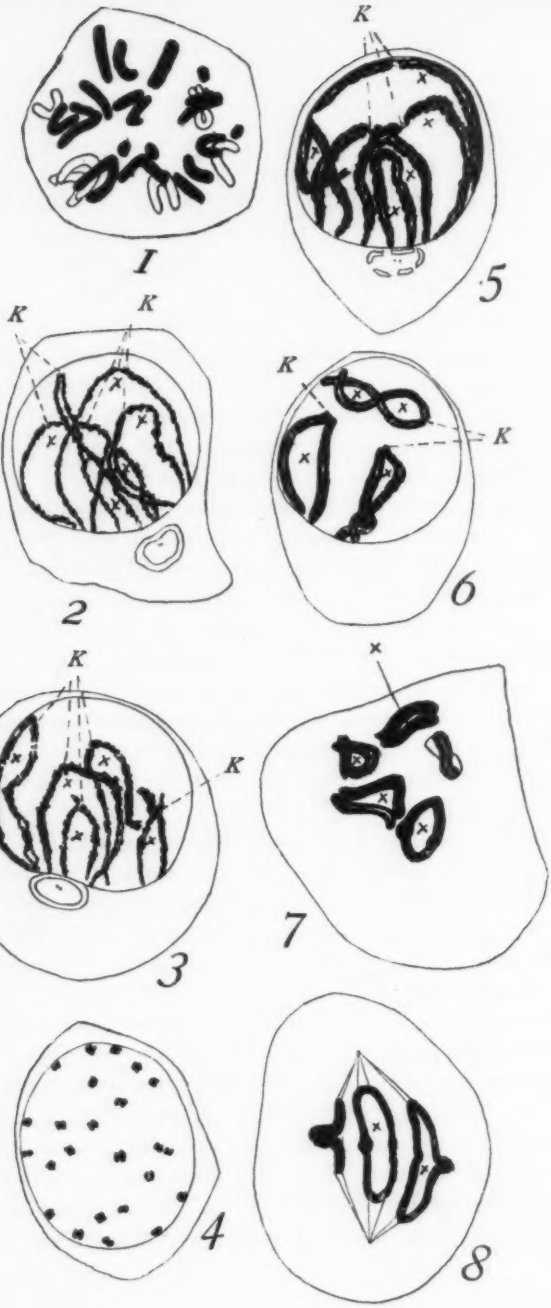
THE HETEROTYPIC MATURATION MITOSIS IN AMPHIBIA AND ITS GENERAL SIGNIFICANCE.

THOS. H. MONTGOMERY, JR.

The marked contradiction between the results of workers on spermatogenesis of vertebrates, and of those on the spermatogenesis of arthropods, has led me to examine the formation of the chromosomes of the first maturation mitosis in urodele amphibia. The question at issue is of course the interpretation of the heterotypic division, for if that is an equational division as generally maintained, then in vertebrates there would be no transverse, or so-called "reduction," division of the chromosomes. Much time and thought has been given to the interpretation of the various ring-shaped chromosomes of heterotypic divisions, *i. e.*, as to whether the space enclosed by the ring represents a longitudinal split of a single chromosome, or a space between two univalent chromosomes. Uncertainty and confusion has resulted, because most of these interpretations have not taken into account the earliest stages in the formation of such chromosomes, which are really the only stages that need critical examination.

The two species in which these decisive periods of the spermatogenesis was studied, are *Plethodon cinereus* (Green) and *Desmognathus fuscus* (Raf.); the maturation mitoses occur in the summer, and the testes were fixed in Hermann's and Flemming's solutions, and stained with iron hæmatoxyline. The spermatogenesis is essentially alike in both; Figs. 1–6 are camera drawings of spermatocytes of *Desmognathus*, and 7 and 8 of *Plethodon*.

The normal number of chromosomes is twenty-four as shown clearly on a pole view of a monaster (equatorial plate) stage (Fig. 1); in this figure four of the chromosomes are cross-sectioned, and one of the others shows very clearly the longitudinal split. In the spermatocytes which result from the last spermatogonic division there is just half this number of chromosomes, namely twelve, the so-called reduction in number taking place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in the synapsis stage of the growth period before the maturation divisions. Now what has to be determined is the changes that occur in these chromosomes in the prophases of the first maturation mitosis. In the early prophases (Figs. 2, 3, 5) these chromosomes always show a definite arrangement; in the figures only a few of the chromosomes are shown in each case, namely those which are seen in their entirety in the plane of the section. There is a distinct polarity of cell body and of chromosomes, and it is the same polarity which I found to obtain in the spermatocytes of *Peripatus:* the nucleus lies in the portion of the cell where there is the least amount of protoplasm, and the sphere (idiozome body) at that side of the nucleus directed towards the greatest protoplasmic mass. For these two poles, as a translation of Rabl's "Pol" and "Gegenpol," I used in the case of *Peripatus* the terms "central pole" and "distal pole," and these terms may equally well be applied to the amphibian spermatocytes. The arrangement in the case of the chromosomes is well shown in the Figs 2, 3 and 5. Each chromosome has the form of a loop like a U or a V with the bend or angle of the chromosome pointing towards the central pole, and the free ends terminating at the distal pole of the nucleus; sometimes at these early stages the two free ends of a chromosome may be applied together so that the whole chromosome has the form of an elongated ring; but generally in these early prophases the U or V shape is the prevalent one.

There are just twelve of these looped chromosomes, half the number of those in the spermatogonia, as may be determined by study of cells cut in a plane at right angles to the axis connecting the central and distal poles. Thus in Fig. 4 (corresponding to the stage of Fig. 3) can be counted twenty-four cross-sections of chromatin threads, every two of which represent the two arms of one of the twelve looped chromosomes. Now the point of great importance is that these early U and V-shaped chromosomes have not arisen by any longitudinal splitting of a single chromosome, for in the very earliest prophases, even earlier than that represented in Fig. 2, they have this shape; therefore the space circumscribed by the two arms of the chromosome does not represent a longitudinal split, but a longitudi-

nal split appears later and then along the axis of each arm (Figs. 3–6). What then is the correct interpretation of each chromosome loop? Each U or V is, first of all, a bivalent chromosome, since they occur in half the number of the chromosomes of the spermatogonia. Second, each arm of one of these bivalent chromosomes represents one of the univalent chromosomes of the spermatogonia. In the case of a bivalent chromosome of the form of a U or V, the two univalent chromosomes are connected together at the angle, that is one end of one chromosome is joined to one end of another chromosome; these points of union are marked in the Figs. 2, 3, 5 and 6 by the letter *K*. Just at this point of junction can be seen in many cases, though not so clearly as in *Peripatus*, a broad connecting linin thread, as in the chromosomes of Fig. 5. When a bivalent chromosome is an elongate ring, as in the case of the left-hand one of Fig. 3, then both ends of both univalent chromosomes are connected. The bivalent chromosomes of *Desmognathus* and *Plethodon* thus represent each two univalent chromosomes joined end to end, and the space between the two arms of a bivalent chromosome is the space between two univalent chromosomes, whether this space be bounded by a chromosome of the form of a U, a V, or an O. This is the space marked in the figures by the letter *x*, and, as the present observations show, does not represent a longitudinal split since it is widest at the earliest stages of the prophase.

The longitudinal splitting of the chromosomes is shown in its commencement in Fig. 3, where certain microsome groups are splitting ahead of others. Fig. 5 shows the stage where this split is most pronounced. Fig. 4 shows the split on cross-sectioned chromosomes. It is still apparent in some of the chromosomes of Fig. 7, but in the equatorial plate stage (Fig. 8) is hidden, to reappear at the anaphase as a longitudinal split of each of the daughter (univalent) chromosomes. This temporary disappearance of the longitudinal split during the equatorial plate stage of the first maturation mitosis has been noted in various objects, and I have described it in detail for *Peripatus* and the *Hemiptera*, as has Korschelt for *Ophryotrocha*. Along the plane of this split the daughter chromosomes divide in the second maturation mitosis, so that the latter is an equation division.

It is then apparent that the split along each arm of a bivalent chromosome, is a longitudinal split of each univalent chromosome, and is a preparation for the second maturation mitosis. It is equally evident that the space marked x in the figures does not represent any longitudinal split, but a space between two univalent chromosomes. Hence in the typical chromosomes of the ring form, as shown in Fig. 8, the space enclosed by the chromosome is the space between two univalent chromosomes, and has nothing to do with the longitudinal split. The thickenings so frequently found upon the rings, as in Figs. 7 and 8, are the points of union of the ends of two univalent chromosomes, as becomes clear from the intermediate stages shown in Figs. 6 and 7, and are not, as generally interpreted, portions of the chromosome where the longitudinal split is least evident. The reader may follow the spaces marked x in the chromosomes from the stage of Fig. 8 back through successive stages to Fig. 2, to be certain of this fact. And it is decisive that this space becomes wider the earlier the prophase, as a comparison of Figs. 8 and 2 shows, and does not lead back to a simple longitudinal splitting.

So the form of the bivalent chromosomes is explained, and the fact established that the heterotypic mitosis, the first maturation mitosis, is not an equation division but separates entire univalent chromosomes, while the second maturation mitosis is equational.

These views are in disagreement with the conclusions of most other workers on amphibian spermatogenesis, because the writers have mostly assumed that the space marked x in my figures, the space enclosed by the ring, is a longitudinal split. Flemming first described the heterotypic mitosis and named it; he overlooked the true longitudinal split in each arm of a bivalent chromosome, and concludes the space enclosed by the definitive chromosome to be the longitudinal split. Vom Rath described in *Salamandra* a reduction division on the basis of tetrad formation with spherical chromosomes, but later observers have demonstrated that he studied abnormal mitoses. Meves essentially corroborates Flemming's interpretations, but he did not note the polarity of the spermatocytes as evidenced in the arrangement of the chromosomes, though it is shown in his Fig. 47, Pl. IV., and hence leaves untouched the question of the origin of the bivalent

chromosomes; he also failed to describe the true longitudinal split in each arm of the chromosomes, though certain of his figures show it indistinctly. McGregor confirmed for *Amphiuma* Meves' account for *Salamandra*, but likewise failed to show how the bivalent chromosomes are produced; his Fig. 7, Pl. IV., shows the true longitudinal split, but it is not demonstrated by his figures that this becomes the space enclosed by the definitive chromosome. Eisen likewise interprets, for *Batrachoseps*, the heterotypic division as an equation division, though his "bouquet stage" is essentially similar in its details to my Fig. 2, and though his results might equally well be interpreted as speaking for a transverse division. Kingsbury's account for *Desmognathus* is clearly a careful study; his Figs. 4 and 5 may be compared with my Figs. 4 and 3; he found the polarity of the chromosomes in the nucleus, saw the true longitudinal split (his Fig. 6), but illustrates no stages to show that this split is connected with the space in the definitive chromosomes. Janssen's account for *Triton* appears to be the most detailed and careful, his Figs. 4, 5 and 32 show appearances of the early chromosomes just as I have found; he describes the synapsis stage where the formation of bivalent chromosomes takes place; he was the first to clearly recognize this stage in the Amphibia, but holds the bivalent chromosomes undergo two longitudinal splittings after their transverse segmentation from a continuous chromatin spirem.

In the ovogenesis of Amphibia, to mention only two more recent studies, Lebrun concludes that both maturation divisions are equational, though more on the basis of a lengthy discussion of the definitive forms of the chromosomes than of a description of the early formation stages. Miss King, for *Bufo*, was unable to determine whether the heterotypic division is transverse or equational.

The whole question is one of a careful interpretation of the early stages of the chromosomes, and the mode of formation of the bivalent chromosomes. The workers on this subject have been satisfied for the most part to know that in the spermatocytes the chromosomes are bivalent, without describing the mode of union of every two univalent chromosomes. In *Desmognathus* there is one true longitudinal split, and besides that what has heretofore

been regarded as a second longitudinal split but what is really the space between the two univalent chromosomes of a pair.

The term "reduction division" was introduced by Weismann, to denote a division that separates entire chromosomes, in contradiction to "equation division" which halves longitudinally each chromosome. Weismann, in his splendid conclusion that such a division would be found to occur, unfortunately assumed that there must be a doubling of the normal number of chromosomes before the maturation. This doubling does not occur, as Boveri and Brauer were the first to demonstrate, but instead there is in the synapsis stage, as I first showed, a union of chromosomes end to end in pairs. This union is not due, as Rückert suggested, to the chromatin spirem breaking into half the somatic number of chromosomes, since there is no continuous *chromatin* spirem in the prophases of the maturation mitosis in any of the objects studied by me, but is a union of chromosomes that were disunited before.

That a separation of entire univalent chromosomes in one of the maturation divisions does occur, is shown by the following studies: Rückert and Häcker for the ovogenesis of Copepoda, finding the separation of entire univalent chromosomes to occur in the *second* division; while Lerat does not decide whether such a division occurs, but points out that it is the *first* division which is heterotypical. In the spermatogenesis of *Gryllotalpa*, by Vom Rath; of Hemiptera, by Henking, myself and Paulmier; of Orthoptera by McClung, Sutton, and Wilcox. The first maturation in the ovogenesis of *Ophryotrocha*, according to Korschelt's account, also separates entire univalent chromosomes, and very clearly the first maturation in the spermatogenesis of *Peripatus* according to my own observations. For the ovogenesis of *Limnæa* this is maintained by Linville, but the chromosomes in this object are unfavorable for decisive interpretation. In Isopoda (*Oniscus*) the first division was shown to be reductional by Miss Nichols. For *Thalassema* and *Zirphæa* the same conclusions were reached by Griffin; and Lillie considers it possible that Griffin's mode of interpretation may be applied to the egg of *Unio*. Van Winiwarter in a very excellent detailed study of these stages in the ovogenesis of mammals (*Homo*, *Lepus*), considers it

probable that one of these divisions is reductional. Schockaert, for the ovogenesis of *Thysanozoön*, finds also a reduction division, in contradiction to the earlier work of Van der Stricht. Finally, Carnoy's earlier work speaks very strongly for the occurrence of reduction divisions.

It is then very humorous to read in a recent paper by Meves: "Ich für meine Person halte es aber, heute mehr als je, für gerechtfertigt, das Vorkommen sogenannte Reduktionsteilungen zu bezweifeln." Herr Meves has the right to hold any opinion he pleases, but he will soon find himself in the small minority where dogmatic assertion takes the place of fair-mindedness. As the pupil of Flemming, Meves must stand by his teacher, to the effect that there occur only equational divisions. Herr Meves has had very little experience in the field of the phenomena of maturation, though he has done excellent work in the study of the formation of the spermatozoön, and in his paper on the spermatogenesis of *Salamandra* overlooked all the phenomena which are decisive in regard to a reduction division. I might add, "ich für meine Person halte Herrn Meves' Resultate unbewiesen zu sein."

In the light of the recent work it becomes apparent that "heterotypic" division is synonymous with "reduction" division; it is heterotypic in being the only mitosis in the germinal cycle in which entire univalent chromosomes are separated in metakinesis. This explains why the shape of the chromosomes in this mitosis differ from all other mitoses; if it were an equation division, why should its chromosomes differ so markedly from those in other equation divisions? "Transverse," "reduction" and "heterotypic" divisions express the same phenomenon in different words, and the nail is driven home when the facts are so clear in amphibian spermatogenesis, in the very cells which were held to disprove the occurrence of reduction mitoses.

In my paper on *Peripatus* I proved that the bivalent chromosomes are produced by the union end to end of every two univalent chromosomes, and not by a splitting of a chromatin spirem into half the normal number of segments. This has since been found by several observers to be the case for other objects. Then in a paper, "The Germ Cells of the Metazoa," I showed

that each bivalent chromosome is very probably formed by the union of a paternal with a maternal chromosome, and interpreted this process as part of the phenomenon of conjugation, as a conjugation of the chromosomes. I showed also that when in the spermatogonia certain chromosomes can be distinguished from the others by peculiarities of size, that such chromosomes pair together in the synapsis; this was the case in *Protenor*, *Peliopelta* and *Zaitha*. McClung (1902) has noted a similar case for *Anabrus*. A few months ago Sutton confirmed these conclusions for *Brachystola*, and on the basis of a careful examination of the chromosomes has reached the important conclusion, that, maternal chromosomes of a certain length uniting always with paternal chromosomes of the same length, the result of the reduction division, in separating such two univalent chromosomes from each other, prevents the retention by either of the daughter cells of two chromosomes representing the same characters. Such a result is a strong confirmation of the brilliant postulates of Weismann.

If it be true that wherever a heterotypic mitosis occurs, it denotes the separation of entire univalent chromosomes, then the old strife of whether a reduction division does or does not take place in the maturation of the germ cells is decided in the affirmative, and we are no longer met by the discrepancy of certain animals with and certain without a reduction division. It rests with the opponents of this view to prove that the heterotypic division is an equation division, and that has not yet been satisfactorily done. And in reaching this conclusion I may state frankly that at the outset of my studies I was fully convinced, almost as much as Herr Meves himself, that the heterotypic mitosis is an equation division; only long observations have shown me that it can only be regarded as a reduction division.

McClung has recently (1902) made an embittered attack upon my studies on spermatogenesis, due in large part to a misunderstanding of my position. He states at various places that my views are conflicting, and that he is unable to harmonize them. Certain serious mistakes I made in my first paper (1898) I took pains to correct in two others (1899, 1901), and in these my position is stated very definitely and without contradictions.

Were he as frank in admitting mistakes, there would be great unanimity.

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UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA,
February 10, 1902.

AN OUTLINE OF THE DEVELOPMENT OF A CHIMÆROID.

BASHFORD DEAN.

The common chimæroid of the west coast of the United States, *Chimæra colliei* Jenyns, was taken in deeper water (50–150 fathoms, sp. gr. 1.027, 55° F.) off the Californian coast and in shallower (5–20 fathoms) in Puget Sound. Near Monterey, in water of about 100 fathoms, eggs about to be deposited were taken from females and incubated in sunken cases; by this means a fairly representative series of embryonic stages was secured.[1] In addition one embryo was obtained from an egg-case taken accidentally on the hook of a trawl line, and a series of hatched young were kindly placed at the writer's disposal by the U. S. National Museum.

Spawning occurs at Monterey throughout spring, summer and fall, and a few eggs were obtained by the Chinese fisherman Ah Tack Lee during the winter. The period of maximum spawning is during August. Two eggs are deposited at the same time. And for several hours at least, at the time of protrusion, they hang freely in the water, the small end of each egg-case attached close to the genital opening. The case here terminates in a single string-like process which passes up the oviduct as far as the capsular gland, and here it is so firmly attached at this stage that it can be lengthened — to the degree of several inches — and shortened like a strand of gutta-percha. Exactly how the egg is deposited is unknown; in any event it appears to be

[1] The writer is greatly indebted to President Jordan and to the directors of the Hopkins Seaside Laboratory, at Pacific Grove, for many courtesies extended him during two summers at the laboratory; and to Dr. Ray L. Wilbur for much generous and skilful coöperation in securing material from the Chinese fisher-people during the years 1897, 1898 and 1899. Dr. Wilbur made numerous trips from San Francisco to Monterey during this time, and to his interest in my work and to his boundless energy I am indebted for many of the later and rarer stages of this interesting fish. To Ah Tack Lee, most skilful and intelligent of local fishermen, I owe my best thanks for his services as a collector. To Mr. Naohidé Yatsu, finally, for much valuable assistance in connection with the preparation of the present paper. His are the drawings from which Figs. 8–12, 17–19 are reproduced.

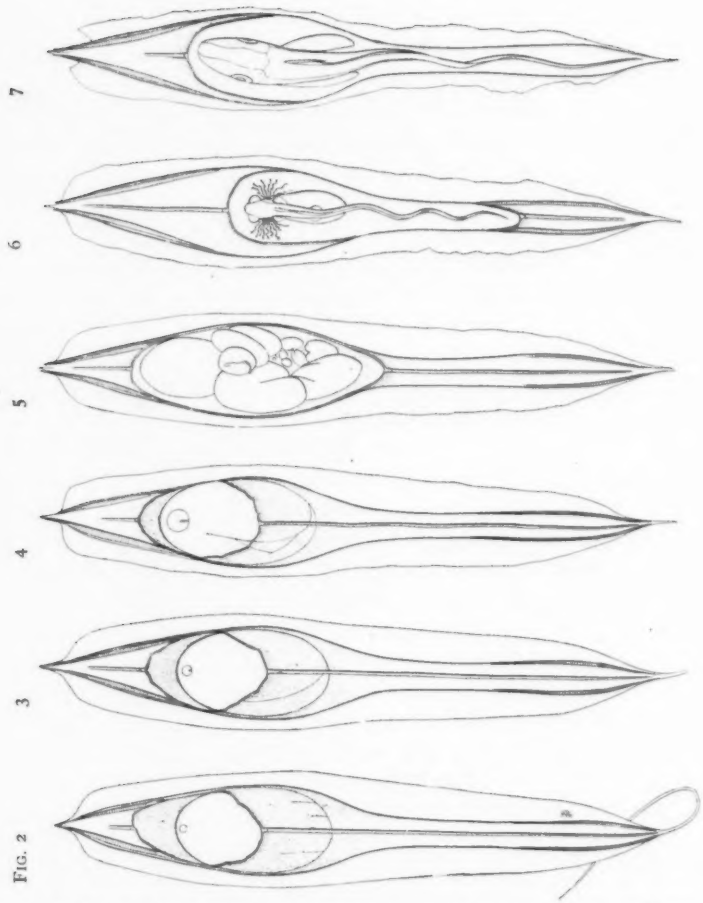
at first attached to stones, etc., by a bulbous tip at the end of the string-like process of the egg-case.

Copulation takes place shortly before the eggs are deposited, for females with eggs in oviducts are usually found with recent marks of the prehensile organs of the male. That the male twists about the female, shark-like, is evident from the character and nearly uniform position of scars near the base of the dorsal fin of the female. These markings, corresponding with the denticles of the frontal organ, indicate that the pair are locked together in copulo. Both mixipterygia appear to be inserted.

There is evidence from experiments with gravid fish kept in aquaria that the elaborate egg-case (Fig. 1) takes but a short time to be formed, possibly not longer than three days. The dilated portion of the case is laid down and is almost perfect, including the lateral processes before the stalk appears; during this time the lining of the oviduct becomes curiously developed to produce the highly specialized structures of the case. The exit-slit or "door" is formed by an abrupt folding in the case's wall. In this folding there is a double row of transversely-directed, interlocking

FIG. 1.

FIG. 1. Egg-case of *Chimæra colliei*. 'Ventral' aspect. × 1. The case is of extraordinary length (about seven inches to the base of the terminal filament) compared with the total length of the female (about twenty-three inches). The egg itself when freshly deposited is elongate and depressed (about 1⅛ in. x ¾ x ½ inches), after the fashion of elasmobranchs. It is of similar syrupy consistency, flowing away as soon as the vitellina is ruptured.



FIGS. 2–7. Characteristic stages in the development of *Chimæra*. These are shown in the opened egg-capsules and represent (2) late blastula, (3) gastrula, (4, 5) two stages of early embryos, (6) a late embryo, and (7) a young fish about to escape from the capsule. The retarded segmentation of the egg is indicated in Figs. 2–5, but in the three earlier stages the cleavage lines cannot be seen through the wall of the egg-capsule (as has been indicated diagrammatically).

lamellæ, and around their delicate tips the thin walls of the shell weather away so that, by the time of hatching, the interlocking[1] lamellæ can disengage and thus permit the young fish to push its way through the folds. The exit-slit of the egg-case is thus a line of rupture. By somewhat similar lamellæ framing a bilateral series of fenestræ ventilation within the case is obtained and perfected in later stages. The fenestræ (as in eggs of certain elasmobranchs) occur in the hinder end of the case, and in latest stages water flows through them, a current being created by the constantly undulating tail of the embryo. The latter has at all times a highly specialized relation to the egg-case, as is indicated in Figs. 2–7. As soon as the embryo can be determined its head points toward the opening-end of the case, the keeled side of the case being dorsal. It is evident that the narrow end becomes the neatly fitted sheath for the elongated tail, and that the widened end fits accurately the greatly enlarged head and trunk. The young hatches as soon as the straight egg-case is filled; thus there is no coiling and wrapping up of the later embryos, as is found, for example, in the case of many sharks.

The Duration of Development.—After the egg is deposited (water temperature 50°–60° F.) fertilization stages continue for about twenty-six hours. If, therefore, we assume that the formation of the egg-case began with fertilization the total duration of this process is probably not less than three days, and not longer than five.

Segmentation (of nuclei), beginning about twenty-six hours after the egg is deposited, lasts from three to four days, each earlier cleavage taking about two hours.

A well-marked blastula is 110 hours old (Fig. 2).

Gastrulation dates from about the eighth day. On the tenth day the embryo resembles Balfour's stage *C* (*Pristiurus*) (Fig. 3); on the seventeenth, *D*; on the nineteenth, *E*; and on the twenty-second, *F* (Fig. 4).

Of later embryos one resembling stage *I* is thirty-one days old (Fig. 5). I have no accurate data of older stages. Assuming,

[1] A row is made up of two sets of interlocking elements, one passing to the right, one to the left, like fingers of interlocked hands.

however, a rate of development proportionate with that of known egg-depositing elasmobranchs I infer that an embryo of five inches can hardly be younger than nine months (Fig. 7). An estimate of a total incubation of twelve months in this species would hardly be excessive.

Fertilization.—Fertilization is shark-like. Polyspermy occurs and a similar number of merocyte nuclei; the conjugation of the pronuclei occurs at a similar niveau in the germinal area, the male pronucleus passing through the germinal protoplasm and then approaching the female pronucleus from a lower plane. Also similar are the location and behavior of the merocyte nuclei during early cleavages. Different from shark, however, is the longer duration of the period of the entrance of the sperm (a newly-entered spermatozoön being present in a preparation showing fusion of pronuclei), and the clearness with which sperm paths are to be noted. Of the latter the surface pits can be seen under low powers. The germinal area is also notably deeper than in the shark.

Segmentation.—As in *Torpedo* (Rückert) and certain other elasmobranchs the appearance of cleavages at the surface of the germinal area is retarded, the first surface furrow appearing about the time of the third or fourth nuclear division. In connection with the furrows at their deeper rim are vacuoles (as in Fig. 8

FIG. 8. Early segmentation. Section through middle of germinal area. At *A* a fissure-like vacuole becomes continuous with an intercellular space. × about 30.

at *A*) which pass into the yolk, and suggest a former condition of deeper cleavage. This inference that the fissure-like vacuoles are to be interpreted as potential but semi-suppressed cleavage spaces is well borne out by the structures of a later stage (Fig. 9) in which the vacuoles are shown to be patently continuous with intercellular spaces, and in which nuclei occur in the underlying germinal masses. It is also to be inferred that a further degree

in the suppression of these vacuolar cleavage spaces would result in a homogeneous and nucleated subgerminal zone. Indeed, in the present material the vacuoles do disappear from the subgerminal zone (Figs. 10 and 11), although they appear as before at the side of the germinal area, and below it, separating masses of the yolk. Note also in this connection the presence

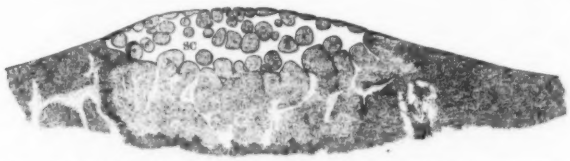


FIG. 9. Early blastula. Vacuoles are shown, continuous in arrangement with cleavage spaces. *SC.* Early segmentation cavity.

of nuclei in the marginal yolk masses thus separated as below the marks * and *, two points in the section, Fig. 11. And in another stage, Fig. 10,[1] it will be seen how far peripherad these nuclei can be traced, *e. g.*, at the points I., II., III., IV. and V. — as far peripherad, in other words, as this region of the egg has been sectioned. It follows accordingly that the yolk mass is separated from the germinal region by no means as abruptly in *Chimæra* as in the allied sharks. For in *Chimæra*,

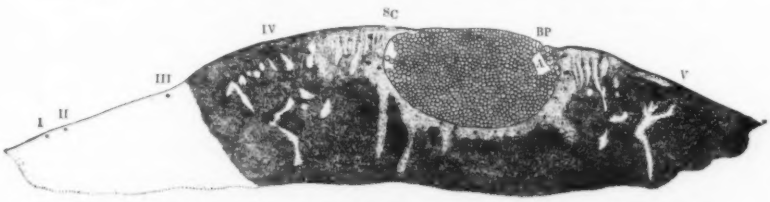


FIG. 10. Early gastrula. Sagittal section. *A.* Archenteron. *BP.* Blastopore. *SC.* Segmentation cavity. I–V. Nuclei lying outside of the germinal area.

as we have seen, vacuoles which in some cases at least represent intercellular spaces pass deep into the yolk region, in the form of more or less vertical fissures. Moreover, again unlike sharks, the yolk nuclei which surround the margin of the germinal area

[1] On one side of the present section nuclei I. and II. occur in the eighth section, and III. and IV. in the seventh and sixth sections respectively; on the opposite side nucleus V. occurs in the fourth section.

do not occur throughout a narrow zone but extend peripherally over a wide area of the yolk. In connection with this remark-

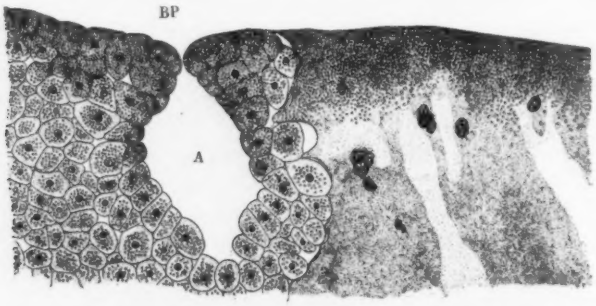


FIG. 10 A. Detail of archenteric region of preceding section.

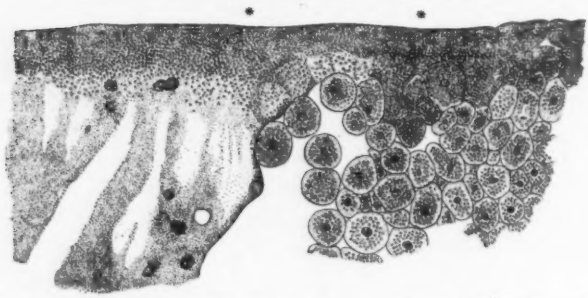


FIG. 10 B. Detail of antero-dorsal germinal wall of preceding section. Observe the cells arising in the overhanging germinal wall below and between the points *—*.

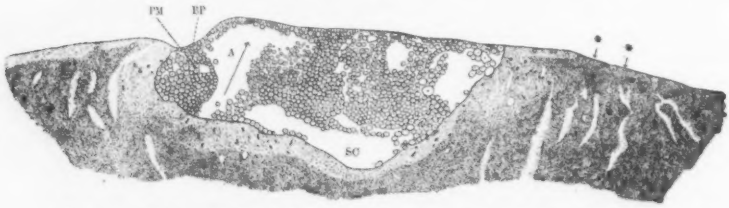


FIG. 11. Early gastrula. Sagittal section. *A*. Archenteron. *BP*. Region of blastopore. *PM*. Posterior cell mass (= ventral lip of blastopore). *SC*. Segmentation cavity. Yolk nuclei are below the asterisks. The arrow denotes the general axis of the archenteron.

able vacuolization of the yolk mass the extension of the nucleus-bearing zone is to be considered *a process of actual fragmentation*

of a large part of the entire egg, a process unique among vertebrates, and suggesting only remotely a physiological parallelism with developmental features in certain lower forms, *e. g.*, digenetic trematodes. Thus in a stage of late blastula the surface view of the egg (Fig. 13), shows with the unaided eye a distinct fissure

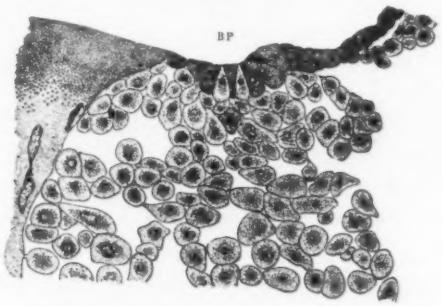


FIG. 11A. Detail of the region of the blastopore of Fig. 11.

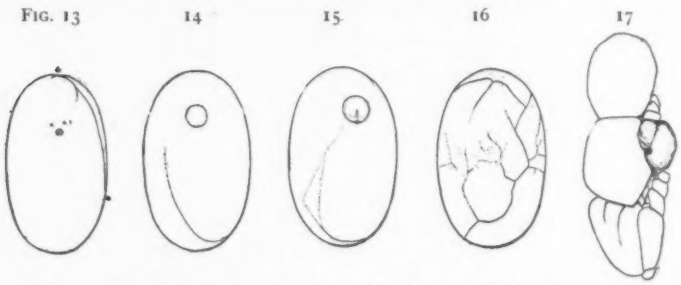
passing near the side of the egg, between the points * and *, and into this run smaller ones.

That this is a fissure and not merely a surface marking becomes clear when the egg is hardened (*e. g.*, in corrosive-acetic), for the sides of the fissure then separate so that one can see between

FIG. 12. Later gastrula. Lettering as in foregoing figure.

them deep into the yolk. In later stages (Figs. 14 and 15) similar conditions maintain, the fissures in these cases, however, extending outward from near the germinal region. In these cases it is found that the ventral side of the egg becomes more completely fragmented than the upper; thus the ventral side of the stage of Fig. 15 shows a variety of surface furrows (Fig. 16),

which by hardening appear as edges of deep fissures. So deeply indeed do these pass into the yolk that the writer was able by means of needles to remove a yolk mass — that lying in the middle of the lower half of the figure — almost entire. This mass, however, broke down in the process of further hardening, and no sections of it could be prepared. In later stages (Figs. 5 and 17) fragmentation continues still further. The embryo with its circumcrescent blastoderm is now attached to a relatively small portion of the yolk; the remainder is broken up into masses, big and little, and in the living egg the smallest are found dissolving into a pasty or creamy liquid, which at first sight is apt to hide the embryo and lead the observer to believe that the egg is dead and addled — an impression he soon loses when he dis-



FIGS. 13–17. Eggs showing the progressive cleavage of the yolk mass. In a blastula (13) a conspicuous fissure is noted between the points * and *. In an early gastrula (14) a fissure extends hindward from near the rim of the blastoderm. In a later stage (15) a progressive fissuring of the yolk mass is seen, anteriorly and posteriorly, and notably in the ventral region (16). In the stage of Fig. 17 the entire yolk mass has undergone division, the blastoderm appropriating a single mass (slightly shaded in the figure).

covers a moving embryo and finds that the fluid is odorless. In ascertaining this the writer may mention, parenthetically, that he was on the point of losing one of his most valuable stages. An egg-case was brought in which had been taken by accident, having become entangled in a trawl line; it was light in weight and its general appearance of wear and tear indicated that it was an empty shell; it was opened, however, and showed a milky fluid which suggested, by analogy of shark eggs, an unpleasantly late stage in decomposition. But as the writer began to pour out the contents of the case carelessly, he saw to his surprise a

mass of bright red gill-filaments. The embryo itself next appeared, as shown here in outline in Fig. 6. It was taken to the laboratory and kept living for half a day; and possibly, like kindred shark embryos, it would have thriven for weeks had one decided to rear it. Corresponding to the mass of yolk attached to the embryos in Figs. 5 and 17, the older specimen had a yolk sac of remarkably small size; the sac was complete, however, and its vitelline circulation resembled closely that of a shark. Furthermore, it may be noted that a young *Chimæra* when newly hatched has no trace of an external yolk-sac. I should record, in this connection, that my friend, Dr. Wilbur, had already (1898) observed on several occasions that the embryo with its blastoderm appropriated only a portion of the entire egg mass, and that the yolk-sac was but a miniature of a shark's. He then believed, like myself, that such a remarkable condition was abnormal. If a normal condition, however, the small yolk-sac and the fragmental yolk bear evidently upon the question of the total segmentation of this yolk-filled egg. And if the evidence is conclusive which the present observations afford, there is here given the first example of a large egg to undergo holoblastic cleavage — an interesting denial of the corollary of 'Balfour's law,' that the quantity of yolk present in an egg determines its holo- or meroblastic character. It may be well, therefore, to review in this connection the evidence of total cleavage in *Chimæra*. (1) The entire egg undergoes a progressive fragmentation, in course of which fissures first pass from the germinal region downward, and finally divide up the egg into a series of yolk masses. (2) The foregoing process is a normal one, having been observed in all specimens (later stages) examined — a dozen or thereabouts. (3) On the evidence of earlier stages distinct fissures (vacuolar) come to be formed in the sub- and circum-germinal yolk, and these are shown to be in many cases continuations of intercellular spaces of the germ itself. (4) The yolk tracts separated by (vacuolar) fissures contain nuclei; this we may conclude from the conditions pointed out in Figs. 10, 10A, and from the presence of nuclei far out beyond the germinal area (Fig. 10). In short, the egg actually undergoes total division, following a nucleation and fissuring of

its yolk-substance. There is of course still the possibility that this total division is not the equivalent of total cleavage in other vertebrates, for it may be due to the action of sperm nuclei — a possibility which finds some support in the subsequent history of the extra-embryonic yolk masses (*v. infra*). This problem, however, cannot be considered specifically at the present time.[1]

Blastula. — A blastula in an early stage is drawn in section in Fig. 9. It shows a mass of blastomeres loosely arranged upon a basis of germinal cytoplasm, traversed by extensive intercellular spaces, the largest of which, *S. C.*, appears by comparison with later stages to represent the cleavage cavity. The topmost blastomeres are closely arranged and somewhat epithelial in character; the lowest are arising from the subjacent germinal well. Particularly interesting is the fissuring (vacuolar) of the subgerminal cytoplasm and of the neighboring yolk, for many of these fissure-like vacuoles are seen to be directly continuous with intercellular spaces, and are, as has above been noted, best interpreted as suppressed (or, better perhaps, retarded) lines of cleavage. Asymmetry is already present in this stage, for it will be seen that upon one side of the figure the blastomeres abut directly against the yolk, and that a more rapid increase in cells is taking place nearer the opposite side of the blastula.

Gastrula. — In Fig. 10, a section in which blastopore and archenteron appear, is represented one of the most valuable stages in my material, for I believe it furnishes us the key to the problem of the gastrulation of *Chimæra* and also probably of sharks. It shows in median section a dense mass of cells lying deep within the germinal area, and closely apposed, save at one point, (anteriormost) to the subgerminal cytoplasm. Near the opposite (posterior) rim of the cell-mass, near the surface, is a small cavity, Figs. 10 and 10 A, *A*, which communicates with the sur-

[1] Another problem which must here be neglected deals with the important relation of yolk nuclei to mesenchyme. In this matter one may note, however, that *Chimæra* confirms Rückert's position as to the conditions in sharks. The confirmation is especially striking, since the additions to the mesenchyme budded out of the germinal wall are here of gigantic size, and cannot be confounded with the neighboring mesoblast cells.

face at the pore, *BP.* Behind it, *i. e.*, between it and the yolk, lie several rows of cells. In this cavity and its surface opening, then, we recognize archenteron and blastopore, and note further that the peculiar pigmentation of the cells of the surface of the gastrula can be followed down on either side, suggesting recent invagination, into the archenteric cavity, half way to its bottom. This, therefore, we conclude, is not a mere intercellular space with a fortuitous opening to the surface, but a definite cavity, whose cells lining the outer half are pigmented. Its cellular wall, moreover, is in general firm and compact, epithelial in character. Accordingly we must admit that in *Chimæra* a gastrula is formed whose blastopore is located not *at* the rim of the early blastoderm but *near* it. And it follows that in this condition there is still retained a phylogenetic stage in elasmobranchian gastrulation wherein the merging of the blastoderm cells into the surrounding yolk has not yet extended to that zone of the blastoderm where the blastopore is forming. It will be observed that growth is taking place at both anterior and posterior margins of the present blastoderm. Cells are coming to be budded off behind the archenteron, as well as at the blastoderm's anterior rim (Fig. 10 B) within the overlying germinal wall itself (*v.* between the points * and *). And even at this late stage blastomeres are found to be budded off abundantly from the subgerminal wall. One notes, further, that the cavity at *SC.* enlarges considerably on either side of the median line and is obviously interpreted as the segmentation cavity. In this stage many merocytes and sperm nuclei occur in the germinal wall.

In Fig. 11 is shown a somewhat later gastrula. Here the compact character of the former stage is lost, the diameter of the blastoderm having doubled. In the anterior region the segmentation cavity, *SC.*, is enlarged and is many-branched. The blastopore is obliterated, owing probably to the backward growth of the cells at the surface of the blastoderm; the archenteron, on the other hand, was greatly increased in size; its anterior wall, spreading out into a thin, somewhat epithelial layer, forms its roof, and its fundus is made up of loose cells, which focus together anteriorly. Behind the archenteron is a mass of cells, *PM.*, read-

ily identified with that in the earlier stage. In an adjoining section, of which a detail is given in Fig. 11A, a rudiment of the blastopore of the earlier stage is shown in the region *BP.*

In a still later gastrula, Fig. 12, the prominent feature is the growth of the blastoderm hindward. The blastoderm has now increased its diameter by about one third, and its surface growth has outstripped that of the subgerminal region — in spite of the fact that the subgerminal wall has notably flattened. Thus it has come about that the posterior region of the blastoderm of the earlier stage has rolled over, as it were, the neighboring germinal wall. The point indicated at *BP.* has thus come to lie first at the edge of the blastoderm, and is next passed under its rim. And as at this point of "invagination" a separation of the cells now occurs, *this may be taken as the reopening of the blastopore.* With this rapid hindward growth the mass of cells, *PM.*, is seen to take a position apparently far forward on the subgerminal wall, but it remains in reality in its previous relative position (*i. e.*, it retains the same actual distance from the anterior border of the blastoderm). Connected again with this backward growth of the surface of the blastoderm it is also evident that the general lie of the archenteric cavity has changed, its anterior end having now been carried backward, its main length (axis of cavity) rotating somewhat as denoted by the arrows in the figures. Other symptoms of the rapid growth of the posterior rim of the blastoderm are seen in its closely compressed cells, epithelial, and in its crumpled condition. Noteworthy, further, is the solidness of the mass of cells roofing the anterior portion of the archenteron, and the reduced size and clearer contour of the segmentation cavity. It will be observed that in this stage the conditions are closely similar to those of the shark, excepting only that the archenteric and segmentation cavities have not clearly merged.

Early Embryos. — Early embryos resemble closely those of typical elasmobranchs (*cf.* Figs. 3, 4, 15); the entire extent of of their surrounding blastoderm, however, is much smaller, and the embryo attains a large size before the adjacent blastoderm surrounds a small mass of the yolk (about three eighths inch in diameter). Stages occur resembling closely sharks in Balfour's notation *C*, *D* and *E*. One might note that the head folds in the

later stages are not as conspicuous. In Fig. 18 is shown a section of an embryo of about stage *C*, which is readily comparable with a corresponding stage of *Torpedo*, as figured, for example, by the Zieglers (*Arch. f. mikr. Anat.*, XXXIX., pl. IV., Fig. 19, VI.); an interesting difference is the depth to which the subblastocœlic entoderm passes into the germinal yolk at the sides of the gut, a feature which might indeed have been expected in this type of egg. In this connection observe also the fissuring (vacuolar) of the germinal yolk.

Late Embryos.—Embryos of about one eighth inch in length can be readily distinguished from those of sharks. The shape of the head is alone distinctive, for the forebrain vesicle sprouts out like a knob or crest and is a feature characteristic of embryos

FIG. 18. Gastrula. Section through the embryonic region of the stage shown in Fig. 14.

up to about three fourths inch in length. Other characteristics are the long delicate tail and the short yolk stalk. In still later embryos external gills are developed, in a very shark-like fashion. A spiracle is present in as late a stage as one and a half inches; it is then a delicate tubular structure and is unprovided with external filaments. Peculiar to the latter is the presence of dilatations, or blood knots, about $\frac{1}{140}$ inch in diameter, brilliantly conspicuous by their scarlet color and large size in the living young. They are doubtless places of multiplication (judging from numerous mitoses) of red blood corpuscles. In this stage, as already stated, the embryo is surrounded by a milk-like fluid, which can only be derived from the continued breaking down of the separate yolk masses of an earlier stage (Figs. 5, 13–17); that it is nutritive there can be no doubt, and there is every reason for believing, judging from the physiological characters of the young of other

elasmobranchs, that it is taken up by the embryo. It is probable that it is appropriated *via* gills (which may become trophonemata, as shown by Wood-Mason, Alcock, Haswell and others), and gut. This stage is of further interest from the standpoint of the morphology of the chimæroid head, for it demonstrates that the autostylous character of the skull is secondary, as had indeed been surmised from the time of Johannes Müller. As indicated in Fig. 19, from a drawing of a wax-plate model,

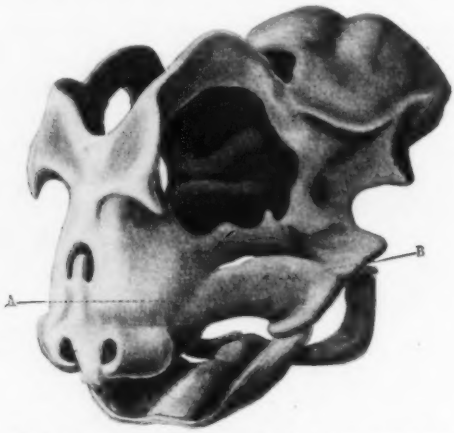


FIG. 19. Skull of embryo shown in Fig. 6. After wax-plate model. The palato-quadrate is shown fusing with the cranium at the points *A* and *B*.

the skull in this stage shows the upper jaw (palate-quadrate) still separate, although even at this early stage its fusion with neighboring cranial cartilage is taking place at both its anterior, and posterior borders, *A*, *B*. Conspicuous in all early stages is the disproportionate size of the anterior and posterior body regions; head and anterior trunk are large, posterior trunk and tail dwindle away narrowly, the latter, however, attaining extreme length. These characters, together with the great size of the eyes in embryonic stages, are clearly in the line of preparing the young fish for the conditions of deep-water living.

Conclusions.—The mode of development of *Chimæra* affords evidence, I conclude, in support of the following theses.

I. That sharks and chimæroids are closely related geneti-

cally. Thus in comparison with other piscine groups, they are allied with one another much as earliest ganoids with the teleosts. Taxonomically we have therefore to revert to Bonaparte's early arrangement (of about 1840) and regard elasmobranchs as a subclass, and selacha and holocephala as natural orders (or superorders).

II. That in some regards, in comparison with selachians, the chimæroid has retained the more primitive developmental features, *e. g.*— the total segmentation of the egg, and the less modified early gastrula. That in other respects it has acquired more highly specialized characters, *e. g.*, restriction of the blastoderm to a smaller region of the egg, appropriation of yolk *via* the external gills (and gut), extraordinary egg capsule and its adaptation to the embryo. (The foregoing conditions, wherein high specialization is found associated with archaic developmental processes, are essentially in keeping with our knowledge of the history of the chimæroid group as derived from anatomy and palæontology. Descended from earliest sharks, this group may well have retained some of their peculiar developmental characters, *e. g.*, in earlier stages; on the other hand new and modified processes of growth doubtless arose in connection with advances which were taking place in the special direction of chimæroid structures.)

III. Of more general significance, I believe, are:

(*A*) The early processes of gastrulation in *Chimæra*, which elucidates the corresponding developmental stage in sharks. In these forms, long studied among vertebrates, gastrulation has been subject to widely different interpretations — indeed in the latest time so careful an observer as Samassa has *even denied the presence in sharks of any process of gastrulation sensu stricto. Chimæra*, it now appears, indicates that the blastopore of sharks is a secondary structure.

(*B*) The accessory mode of nutrition of the late embryo. In sharks the yolk is appropriated by means of a constant extension of the blood-producing area and a progressive differentiation of the vitelline circulation. In *Chimæra*, on the other hand, this mode of nutrition of the embryo is less extensively established, for it has been supplemented by the fragmentation of the yolk

and its appropriation by external gills[1] (and gut). This process is an important one from a larger aspect since it yields a mode of nutriment hitherto unknown in vertebrate embryology — a process by which a late embryo appropriates as food, in the ordinary acceptance of the term, an outlying portion of its own organism.

(*C*) The mode of development of mesenchyme from yolk-nuclei in a somewhat similar way as described by Rückert for selachians.

[1] The origin of the latter process is suggested on the following lines: The gills lying in close contact with the egg came to absorb nutriment from the neighboring finely divided blastomeres and from the interblastomeral fluid. And the embryo came to employ the peripheral yolk more promptly and efficaciously thus than in the ancient way, with the result that the peripheral blastomeres became more loosely associated and finally separated. These melt ultimately into a creamy fluid especially adapted for providing nutriment for the specializing gills of the embryo.